PHARMACOKINETIC INVESTIGATIONS OF ORAL AMODIAQUINE IN GHANAIAN CHILDREN: A CASE STUDY OF SUNTRESO GOVERNMENT HOSPITAL, KUMASI.

By

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DECLARATION

I hereby declare that, this submission is my own work towards the M Pharm (Pharmaceutics) and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgment has been made in the text.

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ABSTRACT.

With the emergence or development of resistance to anti – malarial drugs, the World Health Organization (W.H.O.) now recommends treatment with one of several artimisinin based combination therapies (ACT's) which includes artesunate plus amodiaquine. To date at least fifteen African countries including Ghana have adopted this treatment policy for uncomplicated malaria.

Despite the extensive use of amodiaquine, (as combination therapy) in the treatment of uncomplicated malaria, its pharmacokinetic data, especially in the Sub-Saharan African region is limited. Therefore for optimization of its use in the country there is the urgent need for a clear understanding of its pharmacokinetics. This study therefore seeks to investigate the pharmacokinetics of oral amodiaquine, following administration of the suspension form of the drug to Ghanaian children with uncomplicated malaria. The analysis was based only on urine data.

Fifteen Ghanaian children with uncomplicated malaria, but without any history of liver or kidney diseases and of ages between 8 and 12 years, were made to participate in the study. These subjects or patients who were selected from the Suntreso Government Hospital, were given oral doses of amodiaquine suspension, 10 mg/kg body weight in a single dose study.

Urine samples were serially collected via a non-invasive approach for a period of 30 hrs. Urine concentrations of the drug, in the unmetabolized form were determined. The urine amodiaquine concentration was determined by liquid – liquid extraction (L.L.E.), followed by ultraviolet (U.V) Spectroscopy analysis. The Pharmacokinetic parameters of the drug which were investigated include, fe, kel, $t_{1/2}$, ke, km, ka, and $t_{1/2}a$. Statistically,

the Pharmacokinetic parameter values were estimated at a probability level of p = 0.05. Extremely low fe values were obtained with a range of between 0.0035 and 0.0083; mean, (0.0059 +/- 0.0011). The estimated overall elimination rate constant kel, ranged from 0.1283 to 0.1823 hr^{-1}; mean, (0.1553 +/- 0.0126 hr^{-1}). The corresponding elimination half – life ($t_{1/2}$) range was between 4.0845 and 5.6647 hrs; mean, (4.8746 +/- 0.3691 hrs.).The metabolic rate constant km, ranged from 0.1280 to 0.1816 hr^{-1}; mean, (0.1548 +/- 0.0125 hr^{-1}.), with a corresponding excretion rate constant ke, range of between 0.0004 and 0.0012 hr^{-1}; mean, (0.0008 +/- 0.0002 hr^{-1}.).

An absorption rate constant ka, range values of between 0.3586 and 0.5418 hr^{-1}; mean, (0.4502 +/- 0.0428 hr^{-1}.) were obtained. The corresponding absorption half-life ($t_{½}$ a) values estimated were; range 1.4129 to 2.0271hrs.; mean, (1.7200 +/- 0.1435 hrs).

The study confirms orally administered amodiaquine's rapid absorption as well as extensive hepatic first- pass metabolic effect as published in literature. Statistically, the pharmacokinetic parameters estimated were similar to those published in literature in healthy Caucasian adults as there was no significant difference between the two data. It appears from this observation that, age does not seem to exert any influence on the pharmacokinetics of oral amodiaquine.

However, further statistical analyses revealed high significant differences in the pharmacokinetics of the drug between the study data of Ghanaian children and Zambian adults. The mean half life value and thereby the average plasma concentration at steady state of the drug was significantly higher in the Ghanaian children sub population than in the Zambian adults. This implies, perhaps, the need for separate dosing regimen of oral amodiaquine in these two sub populations. The currently available dosing regimen of the

drug in the country, (which is based on pharmacokinetic studies in East African subjects) upon recommendations from the World Health Organization may thereby be inappropriate. Therefore to optimize oral amodiaquine therapy in the country there may be the need for its dosage regimen adjustment, probably in the downward trend. However, further pharmacokinetic studies based on both urine and plasma data as well as larger study sample sizes across board in Ghanaians, are needed to effect optimization of the dosage regimen of amodiaquine.

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INTRODUCTION AND REVIEW OF RELATED LITERATURE.

1.0. INTRODUCTION.

Malaria continues to be a major threat in the developing world especially in Sub-Sahara Africa, Latin America, and South East Asia. World-wide, there are over one million clinical episodes and three thousand deaths every day. Currently, approximately 40% of the world population resides in areas of active malaria transmission and the disease symptoms are most severe in young children and pregnant women. Despite the fact that malaria is indigenous to most tropical regions, a total of 90% of the disease –associated mortality occurs in Sub-Saharan Africa (Dharmendar et al., 2005).

The only available method for both treatment and prophylaxis of malaria is the use of anti-malarial drugs since a licensed vaccine for malaria has not become a reality (www.jpetaspetjournal.org ,1993). A good number of these anti malarial drugs which have been used in the Sub-Saharan Africa include Chloroquine, Amodiaquine, Sulfadoxine and Halofantrine. Others are Pyrimethamine, Quinine, Primaquine and more recently Artemisinin and its derivatives either in mono or combination therapies (Reynolds, 1996). Chloroquine, the first synthetically developed anti malarial proved to be a magical cure for over thirty years, and was therefore the first line drug in Africa. However, the emergence and subsequent spread of chloroquine – resistant parasites especially plasmodium falciparum strains has made it less effective. This led to the development and introduction of artemisinin – based combination therapy (ACT) as an alternative anti malarial drug of choice in Ghana (www.rollbackmalaria.org {25 October 2006}). Thus, malaria treatment policy shift from chloroquine to artemisinin –

amodiaquine combination was adopted in this country as recommended by the World Health Organization, (Ghana Health Service, 2004).

However, there have been reported cases of adverse effects associated with oral administration of amodiaquine in this country, especially among children. These adverse effects have been suspected to be due to the use of inappropriate dosing regimens of the drug. In spite of the use of amodiaquine in the treatment of uncomplicated malaria, its pharmacokinetic data in children is virtually non-existence in the Sub-Saharan Africa. This study therefore seeks to investigate the pharmacokinetics of amodiaquine suspension following its oral administration in Ghanaian children with uncomplicated malaria.

Research objectives.

To estimate the pharmacokinetic parameters of amodiaquine suspension in Ghanaian children with uncomplicated malaria following oral administration based on urine data.

To compare the estimated pharmacokinetic parameters with published literature values in healthy Caucasian adults and Zambian adults with uncomplicated malaria.

Justification.

Despite the extensive use of amodiaquine, either in mono or combination therapy, its detailed and comprehensive pharmacokinetic data published in literature in children especially in the Sub-Saharan Africa have been lacking or limited. Therefore for amodiaquine's dosage regimen to be effective in this country there is the urgent need for a clear understanding of its pharmacokinetics. Thus further or extensive pharmacokinetic studies are required to improve its dosing regimen and hence the need for the study.

Fifteen Ghanaian children between the ages of 8-12 yrs with uncomplicated malaria but without any history of either kidney or liver diseases participated in the study. These

study patients were selected from the Suntreso Government Hospital, based on among other reasons close proximity, easy accessibility, as well as availability of materials and resources required for the study. Other reasons were higher enthusiasm and maximum co-orperation exhibited by the medical staff, especially the nurses at the children's ward. Finally, the deep interest expressed by the medical superintendent in the study was a major factor for the choice of this medical facility. The site map of Suntreso Government Hospital is shown in Appendix 1, page 101. The analysis was based only on urine data. Pharmacokinetic parameters estimated include the overall elimination rate constant kel, the elimination half life (t ½), and the elimination rate constant of the fraction of administered dose eliminated in the unmetabolized form in urine, ke. The elimination rate constant of the fraction of administered dose eliminated in the metabolized form in urine km, and the fraction of the administered dose that was eliminated in the unmetabolized form in the urine fe, were also estimated. Other parameters calculated were the absorption rate constant ka, and its corresponding absorption half life ($t_{1/2}a$).

Serial sampling of urine from patients, both blank and study after oral administration of drug was the method employed. Thus both blank/control and test/study urine samples were serially voided via a non-invasive approach. These samples were then subjected to Liquid-Liquid Extraction, followed by U-V spectroscopy analyses to obtain the urine concentrations of unchanged or unmetabolized amodiaquine. Further analyses of these data led to the estimation of the pharmacokinetic (PK) parameters enumerated above.

1.1. REVIEW OF RELATED LITERATURE.

1.1.0. OVERVIEW OF MALARIA.

Malaria is the result of infection with protozoa of the genus Plasmodium. The four main species which affect man are; *P. vivax* which causes benign tertian malaria, *P. ovale* which causes tertian fever, *P. malariae* which causes quartian malaria, and *P. fulciparum* which causes malignant tertian malaria which could be fatal if left untreated (Hoffmann). Malaria parasites are transmitted exclusively by the bite of female *Anopheles* mosquito species or by inoculation with an infected blood. The female *Anopheles* mosquito becomes infected when it feeds on human blood containing *gametocytes*, the sexual form of the malaria parasites. *Sporozoites* inoculated by an infected mosquito disappear from human blood within half an hour and enter the hepatic system as *merozoites*. After some days, the *merozoites* leave the liver and invade red blood cells where further asexual cycles of multiplication occur, producing *schizonts*. Tissue *schizonts* generally rupture after 5 to 20 days and release *merozoites*, which invade erythrocytic cells where they multiply rapidly and cause fever, (Erythrocytic infection). The erythrocytes rupture again releasing new generation of *merozoites* into the blood which invade healthy erythrocytes and thereby cause fever whose periodicity depends on the species of parasites. Some *merozoites*, concurrently, develop into male or female *gametocytes*, forming a reservoir of infection for mosquito host (Edwards, 1995).

Treatment of malaria depends on a number of factors among which include the following: severity of infection, age of patient, availability and cost of drugs. Treatment therefore varies and is thereby subject to review. Principal antimalarial drugs used include; 4 – methanolquinolines, (e.g., quinine, quinidine, mefloquine), 4 –

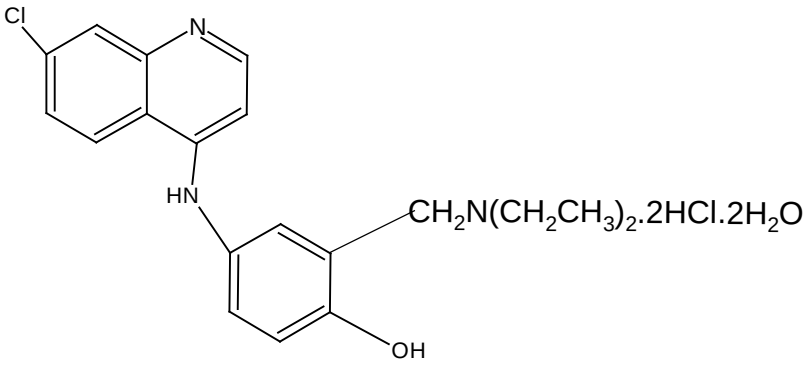
aminoquinolines, (e.g., chloroquine, amodiaquine), 8 – aminoquinolines, (e.g., primaquine), biguanides, (e.g. Proguanil, chlorproguanil), diaminopyrimidines, (e.g., pyrimethamine), 9 – phenanthrenemethanols, (e.g.halofanthrine) and sesquiterpenes, (e.g., Artemisinin and its derivatives, like artesunate, artemeter, arteether, artemos). Other principal groups are sulphonamides, (e.g.,sulphadoxine), antibiotics, (tetracycline, doxycycline), and sulphones, (e.g., dapsone) (Hoffman, 1997).

1.1.1. AMODIAQUINE.

1.1.1.1 Physico – Chemical Properties.

Amodiaquine belongs to the 4-aminoquinoline derivatives and is the dihydrate of 4-(7-chloro-4-quinolylamino)-2-(diethyl amino methyl) phenol dihydrochloride. It contains not less than 98.0% and not more than the equivalent of 101.5% of $C_{20}H_{22}CIN_3O.2HCI$ calculated with reference to the dried substance (B.P., 1980). Amodiaquine is a yellow crystalline powder, odorless, (or almost odorless), with a bitter taste. It is 1 part soluble in 22 parts of water, and 1 part in 70 parts of ethanol (96%). However, it is practically insoluble in benzene, chloroform and ether. A 2% solution in water has a pH of 2.6-4.6 and 1% aqueous solution is 4.0-2.8. It has a melting point of 150-160^{o}C (B.P., 1980). Structural Formula; $C_{20}H_{22}ClN_3O.2HCl.2H_2O$.

Figure 1.1 Chemical Structure of Amodiaquine Hydrochloride.



Molecular Weight; 464.8

1.1.1.2 Indications, administration, and dosage.

Amodiaquine is used principally for the treatment of acute malarial attacks. It is at least, as effective as chloroquine and probably more effective against some chloroquine – resistant strains. It has been used in the treatment of hepatic amoebiasis, lepra reaction, lupus erythematosus, rheumatoid arthritis, and urticaria with variable success. The prophylactic use of the drug is largely restricted owing to acute hepatitis, peripheral neuropathy and irreversible retinopathy(www.amodiaquine.cn {accessed 15 May 2007}. For therapeutic uses, amodiaquine is usually administered either as mono or combination therapy by oral route over a period of three (3) days. However, it can also be administered parenterally via both constant rate intravenous infusion or i.v bolus (i.e. intravenous injection) routes (Looareesuwan et al., 1987).

Adults.

For the treatment of acute malarial attacks: 600mg of the base as a start dose, followed by 200mg after six hours then 400mg daily on each of the subsequent two days is given. In many patients, a single dose of 600mg of the base is often sufficient.

Children.

Treatment dose for children of age 12 years and below is 75mg amodiaquine base daily for three days. For more appropriate dosing, 10mg per kg body weight as a single dose is often sufficient. For prophylaxis the dose is 7mg base per kg body weight which is given once weekly continually for six weeks after the last exposure. Doses may be taken with meals to lessen gastric upsets (www.amodiaquine.cn .{accessed 15 May 2007}).

1.1.1.3. Contraindications.

Amodiaquine is contraindicated in patients with hepatic diseases since it may concentrate

in the liver; hence it must be used with caution in such patients. Children are especially sensitive to 4-aminoquinoline derivatives including amodiaquine. Owing to the narrow margin between the therapeutic and toxic concentrations or levels in children, amodiaquine uses as such must be accompanied with great care or caution. Due to this same reason, amodiaquine must not be administered parenterally in this age group. It is also contraindicated in patients who are renally impaired and hypersensitive to the drug (Winstanley et al., 1987).

1.1.1.4. Tolerability and toxicity.

Oral administration of a single dose of amodiaquine may be followed by abdominal discomfort, nausea, and vomiting. Other side effects include; headache, dizziness, drowsiness, (lethargy), blurring of vision, mental and physical weakness and fatigue. These symptoms are usually mild and transient, and common especially among children. More severe adverse reactions of amodiaquine include; itching, cardiovascular abnormalities, dyskinesia, (impairment of voluntary movement), ocular damage, neuromuscular disorders, and hearing loss. There have been several reports of agranulocytosis, hepatitis, and peripheral neuropathy, and these have limited its uses in prophylaxis (www.amodiaquine.cn , {accessed 15 May 2007}).

1.1.1.5. Antimalarial activity and Mechanism of action.

After oral administration, amodiaquine undergoes rapid and extensive metabolism in the hepatic system, (first pass effect) to desethylamodiaquine (DEAQ). It is most likely that the metabolite, desethylamodiaquine (DEAQ) is responsible for most of the observed anti malarial activity of the parent drug, amodiaquine (www.amodiaquine.cn , {accessed 15 May 2007}) . However, quantifiable levels of amodiaquine in both plasma and urine

are detectable 96hrs after oral administration (Breckenridge et al., 1986).

The mechanism of action of amodiaquine has not yet been determined but since it is a derivative of 4-aminoquinoline and similar in structure and activity to chloroquine, it may have the same mechanism of action as the 4-aminoquinolines. They appear to bind to nucleoproteins and inhibit DNA replication (by inhibiting protozoa DNE gyrase). High drug concentration is found in the malaria parasite digestive vacuoles; and thereby causes the death of the parasite (Hoffman, 1997).

1.1.1.6. PUBLISHED PHARMACOKINETICS OF AMODIAQUINE.

Tables 1.1, 1.2, and 1.3 depict the published pharmacokinetics of amodiaquine under various administration and/or dosage conditions (Krishna et al.,1990).

Table 1.1; Pharmacokinetic parameters of oral amodiaquine 10mg/KgBW in adults.

Table 1.1. Pharmacokinetic parameters of oral amodiaquine (10mg/Kg) in adults.		
Pharmacokinetic Parameter	(Mean +/- s.e.m) values	Range values (95% CI)
C_{max}	31.9 +/- 3.1 ng/ml	28.1 – 35.0 ng/ml
t_{max} (Healthy subjects)	0.5 +/- 0.03 hrs	0.5 – 2.32 hrs
t_{max} (Falciparum patients)	1.75 hrs	
t ½	5.2 +/- 1.7 hrs	1.0 – 9.4 hrs
kel	0.13 hr^{-1}.	0.07 – 1.44 hr^{-1}.
AUC	154 +/- 38 (ng.hr)/ml	
V	38.3 L/Kg	(20.0 – 40.0) L/Kg
CL	5.5 L/Kg/hr	2.0 – 20.0) L/Kg/hr

Table 1.2; P/K Parameters of amodiaquine following i.v. injection of 3mg base/Kg BW over 10mins in seven (7) healthy subjects.

Table 1.2. P/K Parameters of (AQ) following i.v. injection dose in healthy subjects.		
Pharmacokinetic parameters	Mean values	Range values
Cmax	415 ng/ml	(65 – 1921) ng/ml
t ½ α (Distribution phase)	1.7 mins	(0.4 – 5.5) mins
t ½ β (Elimination phase)	2.1 hrs	(0.5 – 5.7) hrs
Vss (Steady state vol. of distribution)	1.74 L/Kg	(2.3 – 95.9) L/Kg
V1 (Central compartment vol.)	1.1 L/Kg	(0.3 – 3.6) L/Kg
CL (Total)	13.0 L/Kg/hr	(4.7 – 56.6) L/Kg/hr

Table 1.3; P/K parameters of amodiaquine following i.v. infusion of 10mg base/Kg BW over 4hrs in ten (10) P. falciparum malaria patients.

Table 1.3. P/K Parameters of AQ following i.v. infusion in P. Falciparum patients.		
Pharmacokinetic parameters	Mean values	Range values
Cmax (Post Infusion)	322 ng/ml	(82 – 836) ng/ml
t ½ α (Distribution Phase)	22 mins	(5 – 126) mins
t ½ β (Elimination Phase)	10.1 hrs	(2.6 – 33.0) hrs
Vss (Steady state volume of distribution)	38.3 L/Kg	(3.7 – 127.9) L/Kg
V1 (Central compartment vol.)	4.6 L/Kg	(0.5 – 29.3) L/Kg
CL (Total)	5.5 L/Kg/hr	(1.6 – 17.3) L/Kg/hr

1.1.2. PHARMACOKINETICS (GENERAL PRINCIPLES)

1.1.2.1 a. Principles of first – order kinetics.

Pharmacokinetics may be defined as the quantitation of the time course of a drug and its metabolites in the body or body fluids, and the development of appropriate models to describe observations and to predict the outcomes in other situations (Roland and Tozer, 1989). The science of kinetics deals with the mathematical description of rate processes or reactions. Typical examples of naturally occurring processes of pharmaceutical interest which conform to first-order kinetics are radioactive decay of materials and the absorption, distribution, metabolism, and excretion [ADME], of drugs in the body. The Pharmacokinetic rate constants are dependent on the concentration or amount of only one component of the system. The kinetics follow first-order or pseudo first-order processes not necessarily because they are so simple but due to the fact that all other components of the system or model except the drug concentration are constant. Thus most in vivo drug processes, especially the [ADME], follow pseudo first-order or first order processes (Banker and Rhodes, 1990).

1.1.2.1. b. Pharmacokinetic working equations

In mathematical terms, the rate law for a first-order process can be expressed in terms of an infinitesimal small change in concentration (dC) over an infinitesimal small time interval (dt) as;

$$Rate = dC/dt = -kC \text{ …….....Eqn. [1].}$$

Where, k is the first - order rate constant.

This is the differential rate expression for a first – order process.

Upon integration, this yields,

$$Ln\ C = Ln\ Co - kt. \text{ ……..Eqn. [2]}$$

But $Ln\ X = 2.303\ Log\ X$, hence;

$$Log\ C = Log\ Co - kt/2.303$$

Equation 2 is the integrated form of the first – order rate law which is linear.

The exponential form of the rate equation for a first-order process is expressed as;

$$C = Co\ e^{-kt} \text{ ……Eqn. [3].}$$

Taking the natural logarithms on both sides of Eqn [3] yields;

$$Ln\ C = Ln\ Co - kt.$$ This is the same as Eqn. [2].

Multiplying both sides of Eqn [3] by V, the total volume of distribution;

$$VC = VCo\ e^{-kt}$$

$$A = DOSE\ e^{-kt} \text{ ……..Eqn. [4].}$$

Rearranging this equation yields;

$A/DOSE = e^{-kt}$.; which is the fraction of the dose remaining at time t.

Where A, is the amount of drug in the body at time t, V is the total volume of distribution, C is the plasma conc. at time t and Co is the initial plasma conc. at time t_o.

- **Half-life ($t_{1/2}$).**

The time required for the plasma concentration (C), to fall to half the original plasma concentration, (C/2), is called the half – life (t ½). For a first – order process this parameter is constant.

Theoretically, a first order process never reaches completion since even the lowest concentration would only fall to half its value in one half – life. For most practical purposes, a first order process may be deemed "complete" if it is 95% or more complete. It has been established that to attain this level of completion at least five half – lives must elapse (Andrew and Leon, 1981). In urinary analysis, total urine collection is effected or deemed complete after at least five half – lives of collection period.

The relationship between half –life ($t_{1/2}$) and rate constant, k, is also a very useful working pharmacokinetic equation and is expressed as;

$$k \cdot t_{1/2} = 0.693; \text{ hence } t_{1/2} = 0.693/k \text{ and } k = 0.693/t_{1/2}.$$

- **Volume of distribution, V.**

The volume of plasma into which a drug distributes in the body at equilibrium is called the total volume of distribution, V. However, the apparent volume into which a drug distributes in the body at equilibrium is referred to as the apparent volume of distribution, Vd. Thus the concentration in plasma, C, achieved after distribution equilibrium is complete is a function of the amount of drug in the body, A (or dose) and the extent of distribution of drug into the tissues, V. Mathematically, this is expressed as;

$$V = A/C, \text{ and at zero time, } Vd = DOSE / Co.$$

Where Co, is the initial plasma concentration at zero time t_o

The total volume of distribution V, may also be defined as the proportionality constant

between the plasma concentration C, and the amount of drug in the body, A.

- **Fraction of dose remaining, (A / DOSE.)**

For a first – order kinetic process;

$A = DOSE \cdot e^{-kt}$; which implies that, $A/DOSE = e^{-kt}$.

Thus fraction of dose remaining in the body, $A/DOSE = e^{-kt}$.

Expressing time relative to half – life, ($t_{1/2}$,) and letting n, be the number of half – lives elapsed after a bolus dose, ($n = t/t_{1/2}$), and $k = 0.693/t_{1/2}$; then the fraction of dose remaining in body can be deduced as follows;

$$A/DOSE = e^{-kt} = e^{-(0.693/t1/2).t} = e^{-0.693n}. \text{ But } e^{-0.693} = 1/2;$$

Hence $A/DOSE = (½)^{n}$…….. Eqn. 5.

Thus ½ or 50% of dose remains in the body after first half – life and ¼ or 25% remains after second half – life and so on (Roland and Tozer, 1989)..

- **Clearance, CL_T.**

This is the proportionality factor or conversion factor which relates the plasma concentration, C, to the rate of drug elimination, dA/dt. Thus,

$$\text{Rate of elimination, } dA/dt = CL_T . C.$$

Mathematically, clearance total is expressed as; $CL_T = k .V$

Owing to the additive concept of clearance, the total clearance, CL_T, can be expressed as the sum of metabolic clearance, CL_M, and renal clearance, CL_R.

$$\text{Thus, } CL_T = CL_M + CL_R. \text{ (Roland and Tozer, 1989).}$$

1.1.2.2. Pharmacokinetic Models.

Drug processes which often occur simultaneously within the body are in dynamic state. In order to describe such a complex biologic system, a hypothesis or model which is based on simplifying assumptions is conceived using mathematical terms. These are a concise means of expressing quantitative relationship concerning the movement or concentrations of drugs in the body. Various mathematical models can be devised to simulate the rate processes of drug absorption, distribution, and elimination. Meanwhile, they make possible the development of equations to describe drug concentrations in the body as a function of time (Andrew and Leon, 1981).

Pharmacokinetic models may be classified into two main categories namely, compartmental/non – compartmental on one hand and physiologic or physiologically – based pharmacokinetic [PB-PK] models on the other hand.

1.1.2.2. a. Compartmental Models.

Compartmental models are based on assumptions using linear differential equations. A compartmental model provides a simple way of grouping all the tissues, (that have similar blood flow and drug affinity), into one or two compartments where drugs move to and from the central or plasma compartment. The compartmental models are particularly useful when there is little information about the tissues. Typical examples of this model include, one – compartment, and multi – compartment models (www.ualberta.ca , {accessed 2 May 2007}).

- **One – compartment open model.**

After intravascular administration, for example i.v. bolus, a drug may distribute into all the accessible regions instantly. Instant distribution of drug in the body may lead to the

consideration of the body as a homogeneous container for the drug and the disposition kinetics may be described as a one compartment open model. The time course of a drug which follows a one – compartment open model depends upon the concentration which was initially administered into the body, Co, and the elimination rate constant, kel.

It must be recalled that $e^{-kel \cdot t}$ is the fraction of dose remaining in the body at time t.

Hence; $C = Co. e^{-kel \cdot t}$

where, C is the concentration of drug in the plasma at time, t. Taking natural logs on both sides of the above expression yields [Eqn. 2].

$$Ln\ C = Ln\ Co - kel \cdot t$$

This is a linear equation, and on a semi – log scale the rate constant kel is estimated as the slope of the straight line that is obtained after a plot of Ln C against time, t. Other pharmacokinetic parameters assessable from such plots following both intravascular doses, such as i.v bolus, and extravascular doses such as oral administration are expressed as follows; $t_{1/2} = 0.693/kel$; $V = DOSE/Co$; $CL_T = V.kel. = DOSE/AUC$.

- **Multi – compartment Models.**

In practice, very seldom will a drug follow a true one – compartment open model. Upon administration, drugs usually distribute into the vascular space and some readily accessible peripheral spaces in a much faster rate than into deeper tissues. In such cases, the drug is being taken out of the vascular system not only via elimination but also through distribution to other tissues (www.ualberta.cn , {accessed 2 May 2007}). In a multi – compartment model, beside elimination, there are distribution processes that are also involved in removing the drug out of the vascular spaces. Consequently, -dC/dt depends upon more than one single first – order processes. On a semi – log scale, the

sum of more than one straight line will be curvilinear. The equation describing a multi – compartment open model will have many exponential phases. For example, a two – compartment model has two exponential phases in its equation; one for distribution, ($Ao.e^{-\alpha.t}$), and another for elimination, ($Bo.e^{-\beta.t}$). Hence the overall equation for the amount of drug, C, in the body at time, t will be;

$$C = Ao.e^{-\alpha.t} + Bo.\ e^{-\beta.t}. \ldots\ldots\ldots\ldots\ldots \text{[Eqn.6]}.$$

Under these conditions, α and β are rate or hybrid constants controlling the rates of distribution and elimination respectively. Ao and Bo are hybrid values representing the respective initial plasma drug concentrations at initial time t_o during the distribution (α) and elimination (β) phases.

There is evidence that after sometime C will become equal to $Boe^{-\beta.t}$, the extrapolated elimination phase, as if $Aoe^{-\alpha.t}$, the residual distribution phase, is reduced to zero. Indeed depending upon the magnitude of α relative to β, (always $\alpha >>>> \beta$), $Aoe.^{-\alpha.t}$, (the residual distribution phase) reduces progressively until it reaches zero. This is when time t, becomes so large and consequently the exponent $e^{-\alpha.t}$ becomes negligible. Then the equation will be reduced to; $C = Bo\ e^{-\beta.t}$. At this time, the concentrations of drug between the vascular and extravascular spaces have reached a pseudo equilibrium phase. From then on the Ln C versus t, relationship will be described by a straight line ($Bo.\ e^{-\beta.t}$).This concept is the basis of "curve stripping" also referred to as method of residuals, which is the common method for identification of compartmental models. After administration of a drug which follows a multi-compartment model, a plot of Ln C against time, t, would result in a curve. Thus the kinetics of such a drug cannot be accurately described by a one – compartment open model. The following sequence

describes the method of identification of the number of compartments involved in a multi – compartment model. (e.g. a two – compartment model.)

i. Make sure the pseudo equilibrium phase has been attained; i.e. the terminal phase is linear. Extrapolate the terminal (linear) portion of the curve, C, to the Y-axis. This is the "elimination" line $Bo.e^{-\beta.t}$; thus line B.

ii. Choose sufficient number of corresponding points on elimination line B and overall concentration curve C. Subtract corresponding B from C to get A, and plot A values against corresponding time t. If the plotted points can be joined with a straight line then line A, is the "distribution" line, $A = Ao.\ e^{-\alpha.t.}$ and the model is a two – compartment model type. On the other hand, if A, turned to be curvilinear, then there are more than two compartments and have to continue stripping until a straight line is achieved. Intuitively, each straight line represents one exponent or one compartment (www.ualberta.cn , {accessed 2 May 2007}).

1.1.2.2. b. Non – compartmental Models.

Non – compartmental models offer a fast and easy way to compute, graph, and analyze the most commonly used pharmacokinetic parameters associated with blood (plasma and serum) concentration – time data. Routes of administration may be oral, rectal, epidermal, or intravenous. Non-compartmental models are also applicable in urinary data analysis. The equations involved in these analyses are referred to as non – compartmental because they do not require curve-fitting or make any assumptions concerning compartmental models. In non – compartmental modeling, the calculation of pharmacokinetic parameters are based on two standard methods of analyses;

a. curve – stripping, or feathering, or method of residuals, to derive the exponential

terms that describe the blood level curve, and;

b. area under the blood level – time curve (AUC), calculations; [the linear and log trapezoidal methods] (www.summitPK.com/eqns , {accessed 28 August 2006}).

- **Table 1.4. Comparison of pharmacokinetic parameters estimation from compartmental and non – compartmental analysis following i.v. bolus doses.**

PARAMETER.	ONE – COMPARTMENT MODEL.	NON – COMPARTMENT MODEL.
CLEARANCE CL_R	k . V	DOSE / AUC.
Volume of distribution (V.)	DOSE / Co	CL_R / k or DOSE/ (AUC.k)
HALF-LIFE ($t_{1/2}$)	0.693 / k	Regression or terminal slope.
Mean Residence Time (MRT).	1 / k	AUMC / AUC

Moment curves are ct, versus time, t plots (Andrew and Leon, 1981).

Where, CL is the clearance; k is the elimination rate constant; V is the total volume of distribution; Co is the initial dose of drug administered; AUC is the area under the blood level-time curve, AUMC is the area under the first moment curve.

1.1.2.2. c. Physiologic /physiologically – based pharmacokinetic (PB-PK) models.

These are models which are based on known anatomic and physiologic data. If the tissue drug concentrations and tissue binding are known, physiologic pharmacokinetic models, which are based on actual tissues and blood flow, describe the data more realistically. Physiologically –based pharmacokinetic (PB – PK) models are frequently used in describing drug distribution in animals, because tissue samples are readily and easily available for assay. On the other hand, tissue samples are often not available for human subjects, and approximations are often made in applying these models to human.

In physiologic models, the size or mass of each tissue compartment is determined physiologically rather than by mathematical estimation. The concentration of drug in the

tissue is determined by the ability of the tissue to accumulate drug as well as by the rate of blood perfusion to the tissue (Andrew and Leon, 1981).

1.1.2.2 d. Multiple dose regimens

- **General principles.**

Drugs are most commonly prescribed on a multiple – dose regimen; thus to be taken on a fixed dose, fixed time interval basis. With multiple dosing, the plasma concentration and the amount of drug in the body fluctuate and accumulate as well with time and thereby rise toward a steady – state or a plateau. Drug accumulates substantially during multiple dosing because elimination from previous doses is not completed before the following dose is administered. Within each dosing interval tau, the amount of drug in the body just after each dose is the maximum (A_{max}), and just before the next dose is the minimum (A_{min}). The average amount within this same interval, τ, is denoted by (A_{av}). In multiple dose regimen, drug accumulation viewed in terms of either maximum or minimum amount in the body continues until the steady – state is reached. At steady-state the amount of drug lost in each interval equals the amount gained, that is the maintenance dose, D_M. Here, the amount of drug in the body at a given time within the interval are the same from one dosing interval to the other (Roland and Tozer, 1989).

For the more general situation in which a drug is administered at a dosing interval, tau,τ, the general equations for the maximum and minimum amounts in the body after the N^{th} dose (A_N,max ; A_N,min) and at steady – state (A_{SS},max; A_{SS},min) are expressed as;

a. Maximum amount in body after N^{th} dose,

A_N, max = DOSE. $(1 - e.^{-Nk\tau}) / (1 - e.^{-k\tau})$……..[Eqn.7].

b. Minimum amount in body after Nth dose,

$A_N, min = A_N, max \cdot e.^{-k\tau}$……[Eqn.8].

c. Maximum amount in body at steady – state.

$A_{SS}, max = DOSE / (1 – e.^{-k\tau})$…….[Eqn.9].

d. Minimum amount in body at steady – state,

$A_{SS}, min = A_{SS}, max \cdot e.^{-k\tau} = A_{SS}, max – D_M$. ……[Eqn.10].

- **The plateau or steady – state calculations.**

The average amount of drug in the body at steady – state, (A_{SS}, av.) is readily calculated using the steady – state concept; average rate- in must equal average rate- out. The input average is ;(F.DOSE) /τ: while the average output is; k . A_{SS}, av. Where

F = bioavailability of the drug, k = elimination rate constant, and

A_{SS}, av. = is the average amount of drug in the body over the dosing interval, tau,τ at steady – state. Hence; Ass, av. = (F.DOSE) / k.τ. This can also be expressed as;

$$Css, av. = (F.DOSE) / V.k.\tau \text{……[Eqn.11].}$$

Where Css, av. is the average plasma concentration over tau,τ, at steady – state.

The inference is that, drug accumulation is independent on the property of the drug; it is rather dependent on the frequency of administration relative to half – life, i.e. $t_{1/2}/\tau$ or $1/k\tau$, [or DOSE/τ] (Roland and Tozer, 1989).

- **Approach to plateau and accumulation index, (Rac.).**

In multiple – dose schedules, the approach to plateau depends on the drugs half – life. Similarly, the degree of drugs accumulation also depends on both the half – life (t ½) and the frequency of administration. This latter factor also determines the extent of fluctuation in the amount of drug in the body at the steady – state. The approach to the

steady – state can be expressed in mathematical terms as;

A_N, max / A_{SS}, max; = A_N, av /A_{SS}, av = A_N, min /A_{SS}, min = $(1 - e^{-Nk\tau})$……..[Eqn.12].

Where A_N, is the corresponding amounts of drug in the body after the N^{th} dose, within the dosing interval tau,τ. Furthermore, if the amounts at steady – state, A_{SS}, are compared to the corresponding values at time t, after the first dose, A_1, then the accumulation index, Rac, would be obtained. Thus;

A_{SS}, max /A_1, max = A_{SS}, min /A_1, min = A_{SS} av./A_1.av = $1/ (1 - e^{-k\tau})$ = Rac…[Eqn.13].

Thus the maximum, minimum, and average amounts of drug at any time within the dosing interval at plateau are Rac multiplied by the values at the corresponding times after the first dose (Roland and Tozer, 1989).

- **Loading and maintenance doses.**

When the first or initial dose is intended to be therapeutic it is referred to as the loading dose, D_L. The dose required to sustain the therapeutic amount in the body on subsequent dosing is the maintenance dose, D_M. In multiple – dose regimen, the initial dose rapidly achieves the therapeutic response, while subsequent doses maintain the response by replacing drug lost during the dosing interval. The maintenance dose, D_M, therefore is the difference between the loading dose, D_L, and the amount remaining at the end of the dosing interval, $D_L.e^{-k\tau}$. Thus;

$$D_M = D_L - D_L.e^{-k\tau}. = D_L (1- e^{-k\tau}) \text{……[Eqn.14]}$$

This implies that; $D_L = D_M / (1 - e^{-k\tau}). = D_M$ **.** Rac

Hence, $D_L/D_M = 1/ (1 - e^{-k\tau})$ = Rac……[Eqn.15].

This equation [15] is generally referred to as “dosage – regimen equation”. The ratio of loading to maintenance dose is equal to the accumulation index and this depends on

the half –life (0.693/k) and the dosing interval, tau,τ (Roland and Tozer, 1989).

- **Dosage – regimen design.**

Dosage regimens are designed to maintain plasma concentrations, Cp, within the therapeutic window which is defined by a lower limit, Cpmin, and an upper limit, Cpmax. The steady-state average plasma concentration, Css, av. as in [Eqn.11] is expressed as;

$$Css, av. = (F.DOSE) / V.k.\tau.$$

A dosage regimen may be designed by setting the dosing rate, (DOSE/τ) either to achieve the steady state average concentration, C_{SS}, av, or to maintain a peak concentration, (Cp, peak). In both approaches, plasma concentrations Cp, are maintained within the therapeutic window throughout the dosing interval,τ (Roland and Tozer).

1.1.3. RENAL ELIMINATION KINETICS. (URINARY ANALYSIS.)

1.1.3.1. Physiological basis of renal excretion.

The major organ for excretion of drugs is the kidney and the basic or fundamental unit of the kidney is the nephron. Within the nephron are three major eliminating processes namely, the glomerular filtration (which occurs in the Bowman's capsule), tubular secretion (which occurs primarily in the proximal section), and tubular reabsorption, which occurs all along the nephron. Active reabsorption if present usually occurs in the proximal section while passive reabsorption is restricted to the distal portion. The net process from the combined three eliminating processes determines the final renal excretion of the drug by the kidney (Roland and Tozer, 1989).

- **Renal clearance, (CL_R.)**

One method of quantitatively describing the renal excretion of drugs is by means of the

renal clearance value, CL_R for the drug. Renal clearance can be estimated as part of the total body clearance for a particular drug, and can also be used to investigate the mechanism of drug excretion. If the drug is exclusively filtered but not secreted nor re-absorbed, then the renal clearance will be about 120ml/min in normal subjects. This is the creatinine clearance value and furthermore, an indication of the glomerular filtration rate (GFR). If the renal clearance value is less than 120ml/min then one can assume that at least two processes are in operation; glomerular filtration and tubular re – absorption. However, if the renal clearance is greater than 120ml/min, then tubular secretion must be contributing to the overall excretion process. It is also possible that all the three eliminating processes are occurring simultaneously (www.boomer.org , {accessed 3 February 2007}).

In mathematical terms,

Excretion rate = CL_R **.** Cp; where Cp is the plasma concentration at time t.

This implies that, CL_R = Excretion rate / Cp……[Eqn.16].

Analogous to the above series of processes within the kidney (nephron), where the net renal excretion rate is determined by the combined three eliminating processes;

CL_R = (Filtration rate, + Secretion rate, + Re – absorption rate.) / Cp.

Renal clearance may attain a value of zero, (0ml/min) the normal value for glucose which is usually completely re – absorbed, with extraction ratio, E, value zero. Renal clearance can also assume the renal plasma flow rate of about 650ml/min, for compounds like p-aminohippuric acid, (PAH), with extraction ratio, E value of (1) unity. These usually, are completely secreted or excreted by the kidney (www.boomer.org , 3 February 2007). For most drugs which are excreted in the unchanged/unmetabolized form, it has been

established that there is a good correlation between creatinine clearance and the drug's clearance or its observed elimination rate constant, kel. [i.e. Dettli plots] (Winter, 1988). Various investigators have developed cohort equations which allow calculation of creatinine clearance CLcr, in a patient or subject using serum creatinine values, CScr. Typical example of wider application is the equation of Cockcroft and Gault. This is expressed as;

Males; CLcr = ([140 – age] **.** body weight) / 72 **.** CScr……..[Eqn.17]

Females; Use 85% of the value calculated for males. (Wagner, 1975).

Renal clearance can be estimated by various methods depending on the available resources and conditions. Some of these methods are briefly enumerated below.

a. Renal clearance may be calculated using the pharmacokinetic parameters ke and V as;

CL_R = ke**.**V……[Eqn.18].

b. Renal clearance can also be calculated by measuring the total amount of drug excreted du, over some time interval dt. Dividing the excretion rate, (du/dt), by the plasma concentration Cp, measured at the mid – point of the time of collection interval, results in CL_R value (i.e. Eqn.16). This is particularly useful in urine sampling/data analysis. Thus, Renal clearance = Rate of excretion (R), / Plasma concentration, Cp; or,

CL_R = (du /dt) / Cp. = R/Cp……[Eqn.16].

c. Renal clearance can also be estimated as the product of the extraction ratio, E, and the plasma or blood flow rate, Q, to the eliminating organ. CL_R = E**.**Q……[Eqn.19].

d. Clearance can also be calculated as the fraction of the total dose administered to the total AUC. This is for data only systems which are non – model dependent. Thus;

CL_R = DOSE / AUC……..[Eqn.20].

1.1.3.2. Estimation of pharmacokinetic parameters using urine data only.

Sometimes it may not be possible to collect blood (plasma) samples but one may be able to estimate the amount of drug excreted unchanged into urine. For instance, it may not be possible to take repeated blood samples from certain patient populations such as peadiatrics. In others, the apparent volume of distribution may be so large that plasma concentrations are too low to be evaluated. Furthermore, lack of sufficiently sensitive analytical techniques can, and has often prevented measurement of the concentration of many drugs in plasma (www.boomer.org , {accessed 3 February 2007}). Under these conditions urinary excretion data becomes more appropriate for pharmacokinetic studies. The usefulness of urinary excretion data in pharmacokinetic studies of drugs may further be more appropriate where non-invasive methods is desirable.

1.1.3.2. a. The scheme for the model

If we collect data for amount of unchanged drug excreted into urine, it may be possible to obtain valuable pharmacokinetic information. In this study, when a one – compartment model analysis is applied to or fitted to the urinary excretion data, we may have two parallel pathways of the overall elimination process. The elimination of the fraction of administered dose excreted in the unmetabolized or unchanged form in urine, is defined by an elimination rate constant ke. The fraction of administered dose which is eliminated in the metabolized form is characterized by an elimination rate constant km. Nonetheless, there are other possible routes of elimination such as air, sweat, and bile metabolism and these are generally considered as shadow metabolism (www.boomer.org , {3 Feb 2007}). Under these conditions the overall elimination rate constant, kel, is related to ke and km by the expression; kel = ke + km. Furthermore, kel is related to fe, the fraction of the

administered dose excreted in the unchanged form by the expression; fe = ke/kel.

1.1.3.2. b. The rate of excretion of unchanged drug eliminated in urine, (du/dt).

Denoting the cumulative amount of unmetabolized drug excreted into urine as, U, then the rate of excretion of an infinitesimal amount of unchanged drug du, over an infinitesimal time dt, (du/dt) may be expressed in terms of ke or CL_R, as;

$du/dt = ke.V.Cp$, which implies that, $du/dt = CL_R.Cp$, as $CL_R = ke.V$.

Where, ke is the excretion rate constant for the fraction of administered dose that is eliminated in unmetabolized/unchanged form in urine.

Substituting for $Cp = Cpo\ .e^{-kel.t}$ in the above equation results;

$$du/dt = ke.V.\ Cpo\ .\ e^{-kel.t}. = ke.DOSE\ .\ e^{-kel.t}. \ldots\ldots \text{[Eqn.21.]}.$$

Taking natural logs on both sides of this equation yields;

$$\ln(du/dt) = \ln ke.DOSE - kel\ .t \ldots\ldots\ldots\ldots\ldots\ldots \text{[Eqn.22.]}.$$

This is the rate of excretion equation of unchanged drug eliminated in urine.

1.1.3.2. c. Cumulative amount excreted as unchanged drug U.

The rate of excretion equation, [Eqn. 21.] is expressed as;

$du/dt = ke.DOSE.\ e^{-kel.t}$, which on rearranging, results in;

$du = ke.DOSE.e^{-kel.\ t}\ .\ dt$. Integrating this equation between time limits zero and t;

$U = ke/kel.DOSE\ .\ [\ e^{-kel.t}]^0.$ – $ke/kel.DOSE\ .\ [\ e^{-kel.t}]^{\ t}$. Analysis of this yields;

$U = ke/kel.DOSE\ .\ [1 - e^{-kel.t}]$. But ke/kel = fe; hence substituting yields;

$$U = fe.DOSE\ .\ [1 - e^{-kel.t}]. \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{[Eqn.23.]}.$$

This is the cumulative excretion equation in urinary data analysis.

1.1.3.2. d. The amount remaining to be excreted (A.R.E.) concept.

Another aspect of the model which can be applied in the current study is the A.R.E

concept. The equation describing this plot is expressed as follows. From [Eqn. 23.];

$U = fe.DOSE.[1 - e^{-kel.t}]$. Substituting $U^{\infty}. = fe.DOSE.$

$U = U^{\infty}.[1 - e^{-kel.t}.]. = U^{\infty} - U^{\infty}.e^{-kel.t}$. Rearranging,

$U^{\infty} - U = U^{\infty}.e^{-kel.t}$, and taking natural logs on both sides;

$Ln\ (U^{\infty} - U) = Ln\ U^{\infty}. - kel.t.$ Substituting $U^{\infty} = fe.DOSE$,

$Ln\ (U^{\infty} - U) = Ln.\ fe.DOSE - kel.t.$ …….. [Eqn.24.].

This is the A.R.E. equation and the term $(U^{\infty} - U)$, is a measure of the amount of drug remaining to be excreted (A.R.E) at time t (www.boomer.org , {3 February 2007}).

1.1.3.2. e. The pharmacokinetic parameters fe, and fm.

The pharmacokinetic parameter fe, is the fraction of administered dose that is eliminated in the unmetabolized or unchanged form in the urine. The parameter fm is the fraction of administered dose that is eliminated or excreted in the metabolized form in the urine. These parameters are of paramount importance and have wider applications in urinary data analysis. These are expressed in the following terms. From equation [23.];

$U = (ke/kel).DOSE.[1 - e^{-kel \cdot t}]$.

As time approaches infinity, U turns to U^{∞} as the term $e^{-kel.t}$ approaches zero; where U^{∞} is the total cumulative amount of drug excreted unchanged at time infinity t^{∞}. Thus,

$U^{\infty} = (ke/kel).DOSE$, which on rearranging, results;

$fe = (ke/kel) = U^{\infty}/DOSE$……[Eqn.25.].

Thus, the parameter fe, can be readily estimated from the urinary excretion data (www.boomer.org , {7 March 2007})

Similarly, for the cumulative amount of drug eliminated in metabolized form in urine, M, the equation for the rate of change of M, with time t is expressed as;

$$M = (km/kel).DOSE.[1 - e^{-kel.t}].$$

In an analogous manner to the above, at infinite time t^{∞}, the total cumulative amount of drug eliminated in the metabolized form in urine, M^{∞}, is given by;

M^{∞} = (km/kel).DOSE, which on rearranging,

fm = (km/kel) =M^{∞}/DOSE……..[Eqn.26.].

According to the mass balance law, the total amount of drug eliminated equals the administered dose. Thus, U^{∞} plus M^{∞} is equal to the dose.

$U^{\infty} + M^{\infty}$, = fe.DOSE + fm.DOSE = (fe+fm).DOSE = DOSE, as fe + fm = 1.

This is however, based on the assumption that information from all the pathways of elimination are available (www.boomer.org , {7 March 2007}).

1.1.3.3. Urinary excretion - time plots or graphs.

Following a fit and subsequent analysis of a one – compartment model to the urinary excretion data, three main analytical plots can be obtained. The plots are the cumulative excretion, rate of excretion, and the amount remaining to be excreted, (A.R.E.) (www.boomer.org , {accessed 7 March 2007})After administration of the drug, urine is collected over finite time intervals and assayed for drug content. Data collected include the volume of urine voided, time interval of collection and the amount of unchanged drug excreted. The data is treated to calculate the following variables; cumulative amount excreted U, amount remaining to be excreted (A.R.E), and the rate of excretion du/dt. Variables so obtained are used to complete the urinary data table which is subjected to further analyses to derive useful pharmacokinetic information. The application of urinary excretion data analyses in pharmacokinetic studies are illustrated by the following case-study involving intravenous injection (i.v) or administration of

150mg of the drug (www.boomer.org , {accessed 7 March 2007})

1.1.3.3. a. The cumulative excretion plot. (U versus t plot).

One convenient way of representing the urine data is by a plot of U, versus time, t; thus the cumulative excretion plot . The equation for this plot, [Eqn.23.] is expressed as;

$$U = DOSE.fe.\ [1 - e^{-kel.t}.].$$

The cumulative excretion-time plot is a mirror image of the amount of drug lost from the body, V.Cp versus time t, plot. As we lose drug from the body it will appear in the urine. The U versus t plot is fairly qualitative and often difficult to get quantitative results directly; however, some important pharmacokinetic parameters can be conveniently estimated. With reference to the case-study whose data is shown in table 1.5, parameters that may be calculated from the U versus t plot include;

Half-life ($t_{1/2}$); this is the $U^{\infty}/2$ corresponding time point value on the curve.

Half-life (t ½) = 3.5 hr. $kel = 0.693/t_{1/2}. = 0.198\ hr^{-1}$.

$fe = U^{\infty}/DOSE. = 99.477/150 = 0.6632$ $ke = kel \cdot fe = 0.1313\ hr^{-1}$.

$km = kel - ke = 0.0667\ hr^{-1}$. $MRT = 1/kel = 5.0505$ hrs.

Table 1.5 Urinary excretion data table for case-study.

Time intervals. (hrs)	Amt. excreted du (mg)	Cum. Amt. excreted U (mg)	A.R.E (U^{∞}- U)	Mid pt. Time (hrs)	Rate of excretion du/dt.
0	0.000	0.000	0.000	0	0.000
2	33.17	33.17	66.31	1	16.59
4	22.20	55.37	44.11	3	11.10
6	14.80	70.16	29.31	5	7.400
8	9.944	80.11	19.37	7	4.972
10	6.636	86.74	12.73	9	3.318
12	4.422	91.17	8.312	11	2.211
18	6.310	97.48	2.000	15	1.052
24	1.998	99.48	0.000	21	0.333

Fig 1.2; Cumulative excretion-time plot for the case-study.

$U = DOSE.fe.[1 - e^{-kel.\ t}.]$

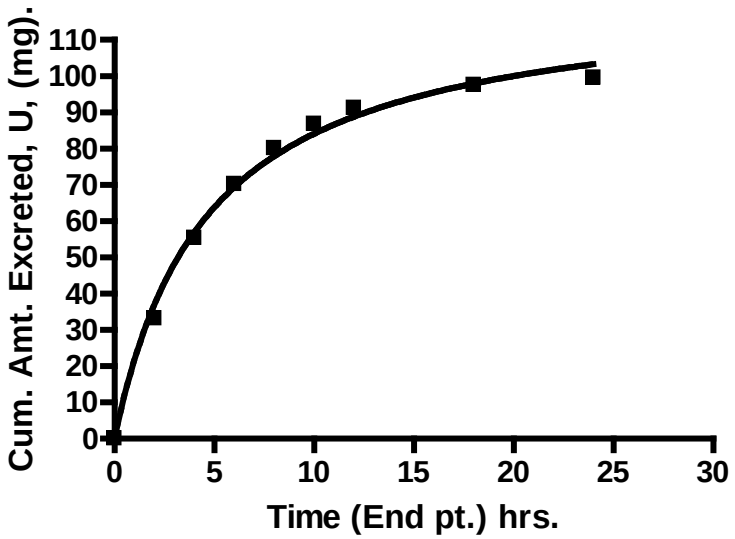


Figure 1.2 is the cumulative excretion curve obtained after a plot of cumulative amount excreted, U versus time t, for the data of the case – study, table 1.5. As the cumulative excretion time approaches infinity, t^{∞}, the cumulative amount excreted value levels off to U^{∞}, which is equal to the product of the dose and fe; (fe.DOSE). Generally, the plot shows U rapidly increasing at first and then approaches a plateau which is U^{∞}. For this approach to be reasonable, it must be ensured that all or total urine is collected. Urine collection must be made for a sufficient period of time to gain an accurate or good estimate of the total cumulative amount of unchanged drug excreted U^{∞}. The period of urine collection must at least be five to six times the half-life. Drugs with long half-life values, for instance in the order of weeks are therefore difficult to be analyzed with this approach. A major disadvantage of this plot is that it only leads to a qualitative measure of the parameters (www.boomer.org , {accessed 7 March 2007}).

1.1.3.3. b.. The rate of excretion plot, (R/E – PLOT).

A second method of urine data analysis, following a fit of one – compartment model to the data, is via the rate of excretion versus time plot, (R/E – plot). From equation [22], the rate of change of the amount of drug excreted into urine, du/dt, is expressed as;

$$\text{Ln } (du/dt) = \text{Ln ke.DOSE.} - \text{kel.t} \quad \text{.....[Eqn.22.].}$$

A plot of ln (du/dt) versus time t, on a semi – log scale yields a straight line with a slope of –kel, and an ordinate intercept of ln ke.DOSE. The approach involves a plot of the average excretion rate against the mid point of the collection time interval on a semi-log scale (Roland and Tozer, 1989). From the urinary excretion data one can calculate the average rate of excretion during each collection time interval; however, the time point for the plot is the mid point time within the collection interval. With reference to the case-study, table 1.5, pharmacokinetic parameters that may be estimated from the R/E-plot include the following.

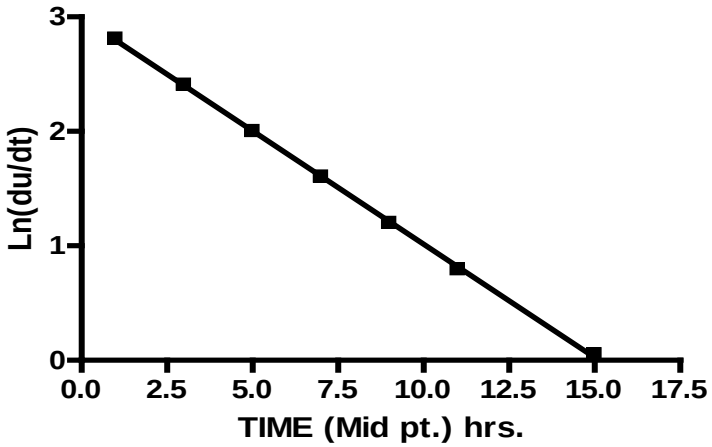
kel = slope = 0.1955 hr^{-1}. $t_{1/2}$ = 0.693/0.1955 (kel) = 3.5448 hrs.

ke = Exp.(2.98) / DOSE = 0.1313 hr^{-1}. fe = ke/kel = 0.6716.

km = kel – ke = 0.0642 hr^{-1}. MRT = 1/kel = 5.1151 hrs.

Figure 1.3 shown below, depicts the excretion rate-time plot for the case study, whose urinary excretion data are shown in table 1.5. Following an i.v. administration of the drug as in this case-study, the R/E-plot results in a straight line with slope –kel, and an ordinate intercept of Ln.ke.DOSE.

Fig 1.3; Excretion rate-time plot for the case-study (i.v admin)
r^2=0.9997; kel=0.1980;Ln ke Dose=2.995



The measured urinary excretion rate reflects the average plasma concentration during the collection interval. The plasma concentration keeps changing continuously within this collection interval. Shortening the collection period reduces the change in plasma concentration but increases the uncertainty in the estimate of excretion rate due to incomplete emptying of the urinary bladder (www.boomer.org , {accessed 7 March 2007}). The urine collection interval, denoted by Δt, is composed of many such very small increments of time. Similarly, the amount of drug excreted in a collection interval is the sum of the amounts Δu, excreted in each of these small increments of time. The major problem with the rate of excretion analysis therefore rests with estimating the excretion rate within the time interval. The average rate of excretion which is directly proportional to the average plasma concentration is therefore employed. Meanwhile, this average plasma concentration is neither the value at the beginning nor at the end of the collection time but at some intermediate point. By assuming that the plasma concentration changes linearly with time, the appropriate concentration is that at the mid point of the collection interval. Since the plasma concentration of drug changes

exponentially with time, this assumption of linear change is reasonable only when loss during the interval is small. Practically, this interval should be less than the elimination half-life of the drug (Roland and Tozer, 1989). A major disadvantage of the procedure is the difficulty in collecting frequent and accurately timed urine samples. The difficulty in collection of urine samples is pronounced especially when the elimination half-life is small. Incomplete emptying of the urinary bladder, within the collection time interval is another source of limitation. Furthermore, the error present in "real" data can obscure the straight line and lead to results which lack precision in this rate analysis (www.boomer.org , {accessed 7 March 2007}).

- **The clearance plot.**

Another parameter which can be estimated from the excretion rate data is renal clearance.

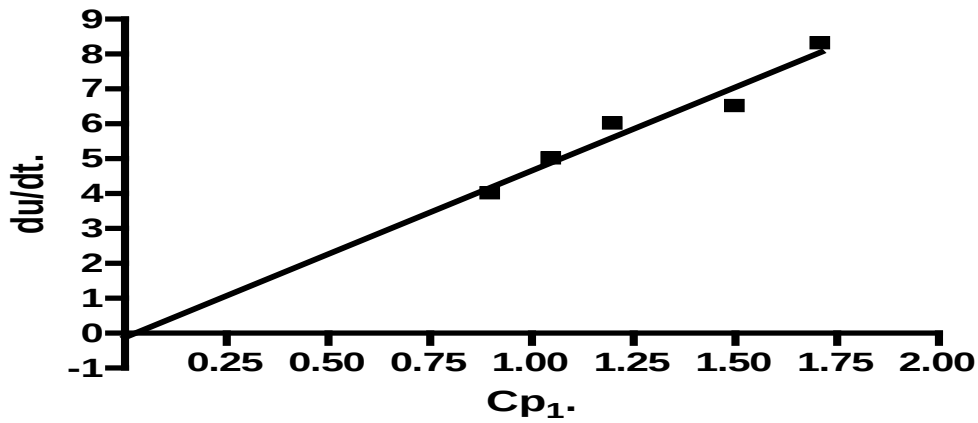
$du/dt = keCp_1.V$; but $CL_R = ke.V$, which on substitution, yields $du/dt = CL_R.Cp_1$.

Where, du/dt = the rate of excretion; CL_R = the renal clearance, and

Cp_1 = the plasma concentration at the mid point time of the urine collection time interval.

A plot of the rate of excretion du/dt, against Cp_1, the plasma concentration at the urine collection interval's mid point time, yields a straight line with a slope of CL_R, (Fig. 1.4), (www.pharmacy.ualberta.ca , {accessed 2 May 2007}).

FIG. 1.4; CLEARANCE PLOT. $r^2 = 0.9474$
Slope=CL_R = 4.780.



If the renal clearance is assumed to be constant, then the average excretion rate becomes directly proportional to the plasma concentration (Roland and Tozer, 1989).

1.1.3.3. c. The amount remaining to be excreted plot (A.R.E. - plot).

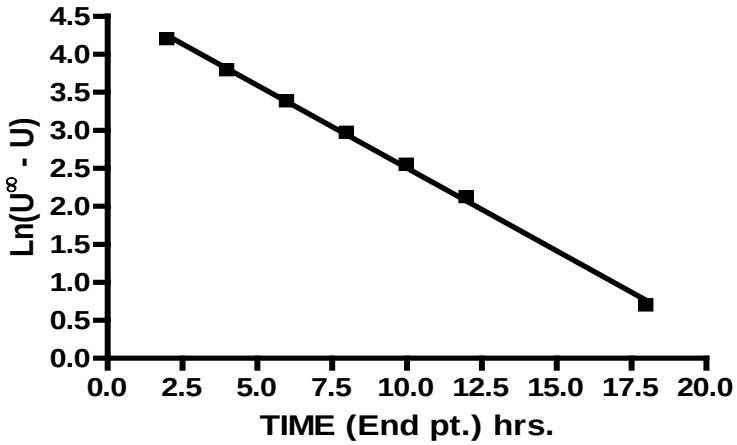
A third analysis of the urinary excretion data which involves a fit of one – compartment model is the amount remaining to be excreted (A.R.E.) plot. The equation, [Eqn.24.] for this plot is expressed as;

$$\text{Ln } (U^{\infty} - U) = \text{Ln fe.DOSE} - \text{kel.t.}$$

The A.R.E. equation is linear; hence a plot of ln $(U^{\infty} - U)$ against time t, on a semi log – scale results in a straight line of slope, -kel, and an ordinate intercept of Ln fe.DOSE The term $(U^{\infty} - U)$ is the amount remaining to be excreted at time t, and if one subtracts U from U^{∞}, at each time point, one would be calculating A.R.E at that time. This type of plot for the case-study data, table 1.5, is shown below in figure 1.5.

(www.boomer.org , {accessed 7 March 2007}).

Fig 1.5; The A.R.E-time plot for the case-study.(i.v. admin)
r^2=0.9985; kel=0.2177; Ln fe Dose=4.6771



Pharmacokinetic parameters that may be estimated from the A.R.E. plot include;

kel = slope = 0.2177 hr^{-1}. $t_{1/2}$ = 0.693/kel = 3.1833 hrs.

fe = Exp (4.677)/DOSE = 0.7163. ke = fe **.** kel = 0.1559 hr^{-1}.

km = kel – ke = 0.0618 hr^{-1}. MRT = 1/kel = 4.5934 hrs.

A major disadvantage with this method of urinary excretion data analysis is that total (all) urine collection is a necessity. Thereby difficulty is encountered in analysis of drugs with long half-lives by this method or approach. Another disadvantage of this approach is that the errors are cumulative, with each collection interval. Hence the total error is incorporated into the U^{∞} value and therefore into each A.R.E value. Furthermore, one missed or lost sample means errors in all the results calculated (www.boomer.org , { accessed 7 March 2007}).

Comparatively, the A.R.E plot tends to smooth out the data (or seems to be easier to construct and analyze) than the rate of excretion plot, (R/E). However, due to the following reasons, the excretion rate plot, (R/E) has wider applications over both the A.R.E. and the cumulative excretion plots (Roland and Tozer, 1989).

(a). Both A.R.E. and cumulative excretion plots require an accurate estimate of the total cumulative amount excreted unchanged, U^{∞}. Hence an underestimation of U^{∞} tends to grossly underestimate the true A.R.E. values as U approaches U^{∞}. This means that there has to be complete urine collection for at least five half – lives. In clinical practice however, this is often difficult to ensure, especially for drugs with long half – life values. The excretion rate method does not require urine to be collected until no more drugs are excreted (i.e. total urine).

(b). Cumulative excretion values, U are usually obtained by summing the amount excreted in each collection interval. Hence assay errors are accumulated while failure to obtain a complete urine collection produces a systematic error in all subsequent estimates of U. Furthermore, a loss or a miss of a single urine sample within collection interval can also lead to this accumulated limitation.

(c).Smoothing out data, as is characterized in A.R.E. analyses can obscure important pharmacokinetic information. Urinary pH and urine flow fluctuate throughout the day and if the renal clearance, for instance, of a drug is sensitive to these factors, it is readily apparent in an excretion rate plot but tend to be lost in the A.R.E. plot.

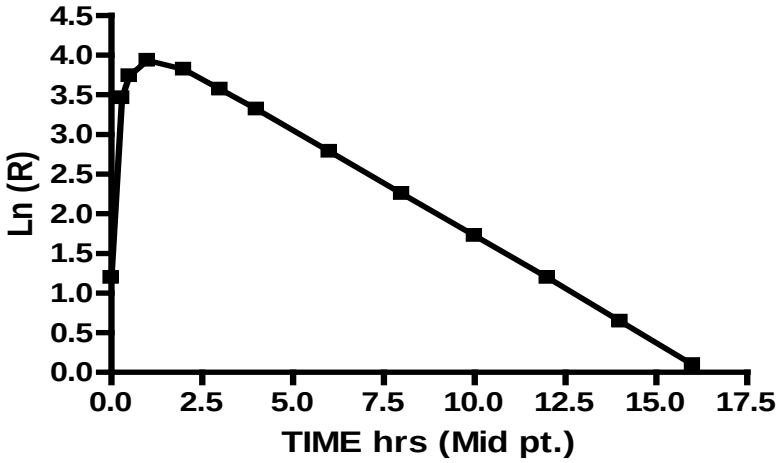
(d). When the drug is administered extravascularly, for example orally, delays in excretion caused by absorption produce distortions of both cumulative excretion and A.R.E. plots, frequently making analysis difficult. In contrast, the excretion rate plot can be readily analyzed (Roland and Tozer, 1989).

For this same reason, absorption kinetics is difficult to estimate using urine samples, especially when the absorption half - life is relatively low. In such a case, absorption would have been completed even before the very first urine sample is voided.

1.1.3.3. d. Non – compartmental model analysis of excretion rate-time data.

Occasionally, it may not be possible to adequately analyze a urinary excretion rate data with a fit of one – compartment model. Under these circumstances a non – compartment model analysis is employed to estimate or calculate the required parameters. The food and drug administration, (FDA) recommends, among others the following parameters for non – compartmental analysis of urinary rate data; R_{max}, and T_{max}. R_{max} is the maximal rate of urinary excretion, and T_{max}, is the time of maximal urinary excretion. These parameters are readily obtainable from excretion rate plots (www.fda.gov.cder , {accessed 16 June 2007}). A case-study of excretion rate, Ln R versus mid point time, t plot following oral administration is shown in figure 1.6 below (www.health.auckland.ac. {accessed 5 March 2007).

Fig. 1.6; Excretion rate-time plot following oral admin. (A case-study) Non-compartmental analysis.



Assuming that renal clearance is constant, then the urinary excretion rate is proportional to the plasma concentration. Hence a plot of average urinary excretion rate against the mid point time simulates a plot of plasma concentration against time. The measured urinary excretion rate reflects the average plasma concentration during the collection

interval. The excretion rate data can therefore be treated in a manner analogous to that of plasma data and estimates of pharmacokinetic parameters can be conveniently calculated from it (Roland and Tozer, 1989). If the excretion rate time course gives some clue about the absorption rate (i.e. excretion rates rise to a peak and then fall), then one can describe the drug absorption process. If a first order input (e.g. oral) is simulated, one can estimate the absorption rate constant ka (www.umanitoba.ca , 2008). The absorption rate constant ka, may be estimated by the method of residuals approach. The overall or terminal elimination rate constant kel, may also be obtained by log-linear regression of the terminal phase of the curve.

CHAPTER TWO

EXPERIMENTAL MATERIALS AND METHODS

2.1. MATERIALS AND EQUIPMENTS.

2.1.1. MATERIALS.

2.1.1.1. CLINIC. (SUNTRESO GOVERNMENT HOSPITAL, KUMASI.)

Fifteen (15) uncomplicated malaria patients; (8-12 yrs.)

Amodiaquine therapy

Urine sample, (blank and test.)

2.1.1.2. REAGENTS

Amodiaquine powder (97 – 102%. w/w.); Fisons lab. Batch No. 3891-384;

Manuf. Date; Sept. 2005; Expiry date; Sept. 2009

Amodiaquine suspension; Pfizer; Batch No. Lot 805; Manuf Date; Aug 2006;

Expiry date; Aug 2010.

Diethylamine, BDH Limited Poole England

Toluene, BDH Limited Poole England

Isopropanol, (Isopropyl alcohol), Merck Germany

2.1.2. EQUIPMENTS

Ultraviolet (U.V) Spectrophotometer, Cecil 3035 (Milton)

Adam Analytical Balance

Refrigerator, Snowcap

General purpose glassware

Whatman’s no.1 filter paper

Separating funnel, (10-mls) and Volumetric flasks

2.2. METHODOLOGY

2.2.1. Sampling of urine, (blank and study samples.)

(a). Blank urine samples.

Fifteen (15) uncomplicated malaria patients, children of ages between 8 and 12 years, with no history of liver or kidney diseases, were recruited into the study. Prior to amodiaquine administration to the patients, blank urine samples were collected overnight. This was used for the preparation of control/standard samples for the analysis. Accurately, 150mg of pure amodiaquine powder (purity; 99.50% w/w), was weighed and dissolved in drug – free blank urine to produce 100ml solution of strength 0.15%w/v. Various solutions of this stock solution of amodiaquine in drug – free blank urine, were prepared by employing the chemical analytical relationship C.V = k. Where C is the concentration, V is the volume and k is the proportionality constant. Preparation of a series of control urine samples of concentration range between 0.003 – 0.00125% w/v was made. The control/standard samples were used to construct a calibration curve.

(b). Test urine samples.

After oral administration of amodiaquine, which was based on 10mg/kg body weight (or 150mg single dose) regimen, serial sampling of test urine from patients was conducted. Thus, test/study urine samples were carefully and serially collected over a period of 30 hrs; (approximately six half – lives). Results from urine collection were recorded and tabulated in urinary excretion data table (www.boomer.org , {accessed 3 Feb 2007}).

2.2.2. Urine samples treatment.

Both blank and test urine samples were frozen immediately after collection and kept at approximately 4^{o} Celsius in a refrigerator until analysis (Segeja et al., 2006).

2.2.3. Liquid – Liquid Extraction, (L.L.E.) – Ultraviolet (U.V) spectroscopy analysis.

The liquid – liquid extraction technique, (L.L.E.) was employed in the extraction of amodiaquine component from the urine sample (Biomed Life Science, 15 January 2003). Ten (10mls) of the urine samples (both control and test) was pipetted and transferred into a 125mls separating funnel. A solvent system made up of diethylamine-toluene-isopropyl alcohol (1:4:5 v/v/v), was used to extract the amodiaquine component from the urine sample (Segeja et al., 2006). Thus the amodiaquine component in the urine sample was extracted with two successive 5mls portions of the solvent system into a 10mls volumetric flask. The combined extract was made up to the 10 – ml mark with the solvent system and then scanned using the U.V – spectroscopy technique for assay of amodiaquine content (www.delloyd.50megs.com , {accessed 15 May 2007}). The extracts were analyzed at a wavelength of 340nm, which is the wavelength of maximum absorbance for amodiaquine, λmax. Absorbance values obtained for standard/control samples were used to draw the calibration curve and hence the derivation of its equation. This equation was used in conjunction with absorbance values for test samples to determine the amodiaquine concentration in the test urine samples. The concentration values were used to complete a urinary excretion data table which was subsequently employed for the rest of the pharmacokinetic analyses and/or investigations (www.boomer.org , {accessed 3 February 2007}).

CHAPTER THREE

3.1. RESULTS.

For the purpose of this study, the patients enrolled at the Suntreso Government Hospital, Kumasi, were coded. The profile of patients selected is detailed in table 3.1 below.

Table 3.1. Profile of uncomplicated malaria patients selected from Suntreso Hospital

CODE	NAME	AGE	GENDER.
001	Kwasi Asare	12	Male
002	Florence Owusu	12	Female
003	Nana Antwi Kwame	8	Male
004	Yaw Boakye.	10	Male
005	Monica Agyemang	12	Female
006	Kwame Asante.	8	Male
007	Matilda Frimpong.	11	Female
008	Beatrice Osei	10	Female
009	Mary Nkansah	9	Female
010	Betty Konadu	11	Female
011	Kojo Agyemang	9	Male
012	Kwame Asiedu	10	Male
013	Agnes Opoku	9	Female
014	Gabriel Wiredu-Mensah	11	Male
015	Felicity Amoah	8	Female

3.001a. Data for patient 001

Table 3.001a. Control samples absorbance data table.

CONTROL SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
1.25	0.326	0.327	0.327
1.50	0.456	0.455	0.456
2.00	0.551	0.551	0.551
2.50	0.664	0.664	0.664
3.00	0.782	0.781	0.781

This data was used to draw the calibration curve, figure 3.001a.

Table 3.001b.Test samples absorbance data table.

TEST SAMPLE CONC. Xμg/ml.		ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
T1	8.0785	2.030	2.030	2.030
T2	11.7186	2.920	2.910	2.920
T3	5.7022	1.448	1.449	1.449
T4	0.2667	0.119	0.120	0.120
T5	0.1481	0.091	0.091	0.091
T6	0.3853	0.149	0.149	0.149

Table 3.001c. Urinary excretion data table for patient 001.

Test samples	Time interval dt (hrs).	Urine vol. (mls).	Urine conc. μg/ml.	Amt. excreted (du)	Cum. Amt. excreted U.	Mid pt. time t.	Rate of excretion du/dt.	A.R.E U^{∞}-U.
T1	0 – 4	50	8.0785	405.0	405.0			1585.8
	4.00					2	101.25	
T2	4 – 8	48	11.7186	561.6	966.6			1024.2
	4.00					6	140.40	
T3	8 – 12	74	5.7022	421.8	1388.4			602.4
	4.00					10	105.45	
T4	12 – 16	500	0.2667	350.0	1738.4			252.4
	4.00					14	87.50	
T5	16 – 20	261	0.1481	104.4	1842.8			148.0
	4.00					18	26.10	
T6	20 – 24	148	0.3853	148.0	1990.8			0.0
	4.00					22	37.00	

fe = U^{∞}/DOSE = 0.0066

3.001b. URINARY EXCRETION-TIME PLOTS FOR PATIENT 001.

Fig 3.001a. Calibration curve for patient 001. r^2=0.9807
m=0.2445;c=0.0548; Y=0.2445X + 0.0548

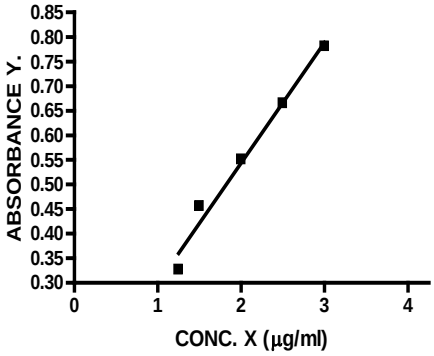


Fig 3.001b. A.R.E Plt. for patient 001.
r^2=0.9888; kel=0.2008; fe=0.0093

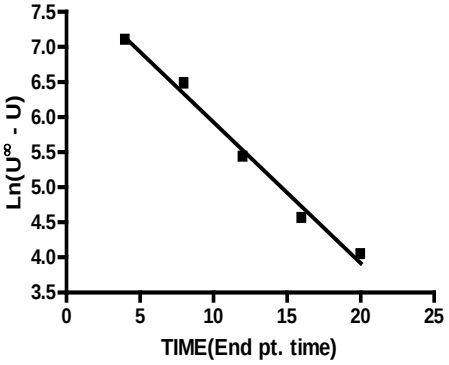


Fig 3.001c. Excretion rate plot for patient 001.

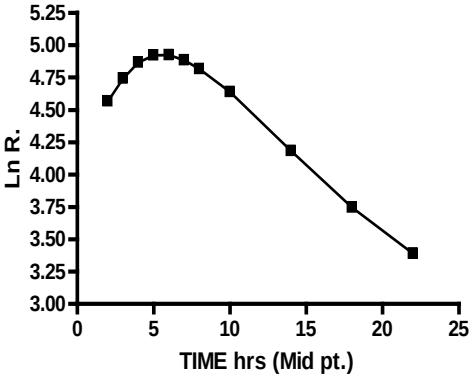


Fig 3.001d. Residual plt for patient 001;
Residual slope=ka=0.6312; $t_{1/2}$a=1.0979; (r^2=0.9666)
Terminal slope=kel=0.1041; $t_{1/2}$=6.6571; (r^2=0.9994)

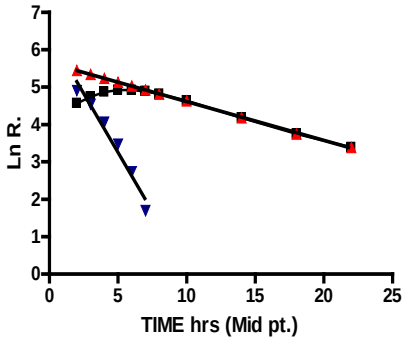


Table 3.001d. Residuals data table for patient 001.

Time	R	Ln R	Ln R^{last}	R^{last}	R^{last} - R	Ln(R^{last}-R)
2	96.1587	4.566	5.4448	231.551	135.3923	4.9082
3	114.6633	4.742	5.3407	208.6587	93.9954	4.5432
4	129.8007	4.866	5.2366	188.0297	58.229	4.0644
5	137.1397	4.921	5.1325	169.4402	32.3005	3.4751
6	137.2769	4.922	5.0284	152.6885	15.4116	2.7351
7	132.1582	4.884	4.9243	137.593	5.4348	1.6928
8	123.4702	4.816	4.816			
10	103.2342	4.637	4.637			
14	65.4967	4.182	4.182			
18	42.3513	3.746	3.746			
22	29.6363	3.389	3.389			

3.002a. Data for patient 002

Table 3.002a. Control samples absorbance data table.

CONTROL SAMPLE CONC. Xμg/ml.	ABSORBANCE (Y1)	ABSORBANCE (Y2)	AVERAGE ABSORBANCE (Y.)
1.2500	0.157	0.158	0.158
1.5000	0.210	0.209	0.210
2.0000	0.261	0.260	0.261
2.5000	0.327	0.328	0.328
3.0000	0.371	0.370	0.371

This was used to draw the calibration curve, fig. 3.002a.

Table 3.002b. Test sample absorbance data table for patient 002.

TEST SAMPLE CONC. Xμg/ml.		ABSORBANCE (Y1)	ABSORBANCE (Y2)	AVERAGE ABSORBANCE (Y.)
T1.	0.1212	0.035	0.034	0.035
T2	0.4264	0.051	0.051	0.051
T3	0.1045	0.033	0.033	0.033
T4	0.2185	0.055	0.055	0.055
T5	0.0711	0.029	0.030	0.030
T6	0.0209	0.023	0.023	0.023

Table 3.002c. Urinary excretion data table for patient 002.

Test samples	Time interval dt (hrs).	Urine vol. (mls).	Urine conc. μg/ml.	Amt. excreted (du)	Cum. Amt. excreted U.	Mid pt. time t.	Rate of excretion du/dt.	A.R.E U^{∞}-U.
T1	0 – 3	195	0.1212	23.634	23.634			109.599
	3					1.5	7.878	
T2	3 – 6	187	0.4264	37.235	60.869			72.364
	3					4.5	12.4117	
T3	6 – 9	240	0.1045	25.080	85.949			47.284
	3					7.5	8.36	
T4	9 – 12	100	0.2185	21.850	107.799			25.434
	3					10.5	7.2833	
T5	12 – 15	240	0.0711	17.064	124.863			8.360
	3					13.5	5.688	
T6	15 – 21	400	0.0209	8.360	133.223			-
						18.0	2.7867	

$fe = U^{\infty}/DOSE = 0.0024$

3.002b. URINARY EXCRETION-TIME PLOTS FOR PATIENT 002.

Fig 3.002 a. Calibration curve for patient 002;r^2=0.9829
m=0.1196; c=0.0205;Y=0.1196X + 0.0205

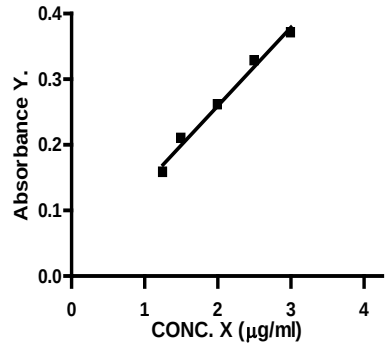


Fig.3.002b. A.R.E Plot for patient 002.
r^2=0.9495, kel=0.2064; fe= 0.0016

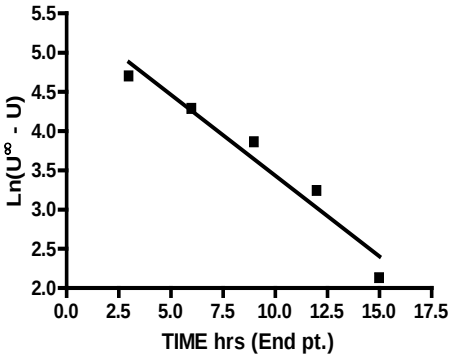


Fig 3.002c. Excretion rate plot for patient 002.

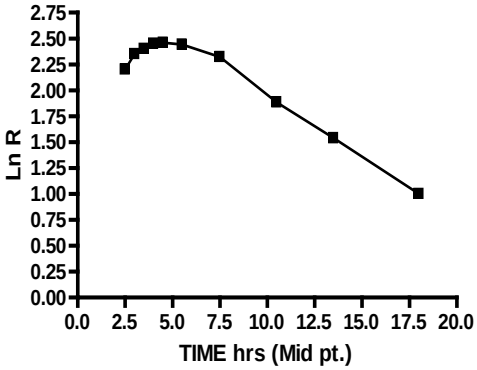


Fig 3.002d. Residuals plot for patient 002.
Residuals slope=ka=0.5411, $t_{1/2}$a=1.2807, (r^2=0.9850)
Terminal slope=kel=0.1247, $t_{1/2}$=5.5573, (r^2=0.9995)

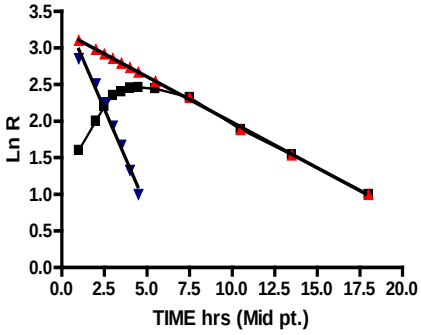


Table 3.002d. Residuals data table for patient 002

Time	R	Ln R	Ln R^{last}	R^{last}	R^{last}-R	Ln (R^{last}-R).
1.0	4.9530	1.60	3.1053	22.3159	17.3629	2.8543
2.0	7.3891	2.00	2.9806	19.6996	12.3109	2.5105
2.5	9.0250	2.200	2.9183	18.5100	9.4850	2.2497
3.0	10.4856	2.350	2.8559	17.3901	6.9045	1.9322
3.5	11.0232	2.400	2.7936	16.3397	5.3165	1.6708
4.0	11.5883	2.450	2.7312	15.3513	3.763	1.3252
4.5	11.7048	2.460	2.6689	14.4241	2.7193	1.0004
5.5	11.496	2.442	2.5442			
7.5	10.2062	2.323	2.323			
10.5	6.5929	1.886	1.886			
13.5	4.6553	1.538	1.538			
18.0	2.7183	1.000	1.000			

3.003a. Data for patient 003

Table 3.003a. Control samples absorbance data table.

CONTROL SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
1.00	0.550	0.551	0.551
1.25	0.623	0.623	0.623
1.50	0.628	0.628	0.628
2.00	0.748	0.747	0.748
2.50	0.941	0.942	0.942
3.00	1.034	1.033	1.034

This data was used to draw the calibration curve, figure 3.003a.

Table 3.003b.Test samples absorbance data table.

TEST SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
T1; 0.0083	0.021	0.020	0.021
T2; 0.1367	0.058	0.057	0.058
T3; 3.2684	0.814	0.814	0.814
T4; 6.7730	1.660	1.660	1.660
T5; 0.4608	0.028	0.028	0.028
T6	-	-	-

Table 3.003c. Urinary excretion data table for patient 003.

Test samples	Time interval dt (hrs).	Urine vol. (mls).	Urine conc. μg/ml.	Amt. excreted (du)	Cum. Amt. excreted U.	Mid pt. time t.	Rate of excretion du/dt.	A.R.E U^{∞}-U.
T1	0 – 4	229	0.0083	1.9007	1.9007			656.0726
	4					2	0.4752	
T2	4 – 8	408	0.1367	55.7736	57.6743			600.299
	4					6	14.4186	
T3	8 – 12	67	3.2684	218.983	276.657			381.3162
	4					10	54.7457	
T4	12 – 16	28	6.7730	189.644	466.301			191.672
	4					14	47.401	
T5	16 – 24	416	0.4608	191.672	657.973			-
	8					20	23.959	
T6								

$fe = U^{\infty}/DOSE = 0.0044$

3.003b. URINARY EXCRETION-TIME PLOTS FOR PATIENT 003.

Fig 3.003a;Calibration curve for patient 003. r^2=0.9803; m=0.2414;c=0.0254; Y=0.2414X + 0.0254

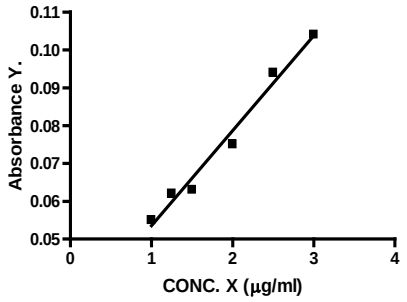


Fig 3.003b:A.R.E plt for patient 003; r^2=0.9046;kel=0.1036;fe=0.0077

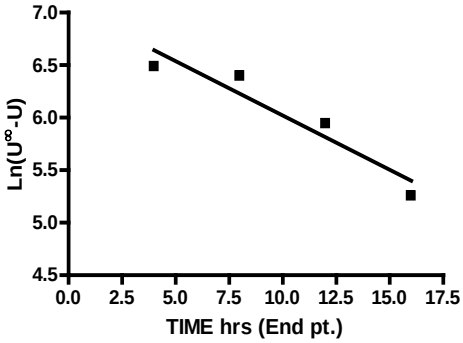


Fig 3.003c; Excretion rate plt. for patient 003.

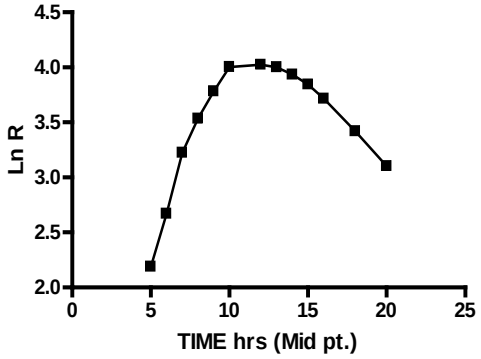


Fig 3.003d; Residuals plt for patient 003;
Residual slope=ka=0.4111,$t_{1/2}$a=1.6857, (r^2=0.9572)
Terminal slope=kel=0.1411, $t_{1/2}$=4.9114, (r^2=0.9994)

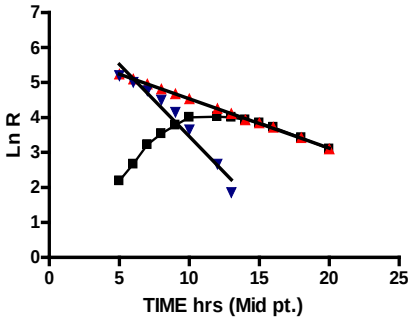


Table 3.003d. Residual data table for patient 003.

Time	R	Ln R	Ln R^{last}	R^{last}	R^{last} – R	Ln(R^{last} – R)
5	8.9263	2.189	5.240	188.6701	179.7438	5.192
6	14.4255	2.669	5.099	163.858	149.4325	5.007
7	25.1284	3.224	4.958	142.3089	117.1805	4.764
8	34.295	3.535	4.817	123.5938	89.2988	4.492
9	43.9477	3.783	4.676	107.3399	63.3922	4.149
10	54.7622	4.003	4.535	93.2235	38.4613	3.650
12	55.9244	4.024	4.253	70.316	14.3916	2.667
13	54.7075	4.002	4.112	61.0687	6.3612	1.850
14	51.1110	3.934	3.934			
15	46.7587	3.845	3.845			
16	41.0997	3.716	3.716			
18	30.5694	3.420	3.420			
20	22.2647	3.103	3.103			

3.004a. Data for patient 004.

Table 3.004a. Control samples absorbance data table.

CONTROL SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
1.00			
1.25	0.355	0.354	0.355
1.50	0.451	0.450	0.451
2.00	0.534	0.533	0.534
2.50	0.679	0.678	0.679
3.00	0.780	0.780	0.780

This data was used to draw the calibration curve, figure 3.004a.

Table 3.004b.Test samples absorbance data table.

TEST SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
T1; 1.6134	0.456	0.455	0.456
T2; 1.6975	0.476	0.475	0.476
T3; 2.3950	0.642	0.642	0.642
T4; 3.2353	0.842	0.841	0.842
T5; 1.9412	0.534	0.534	0.534
T6; 0.8824	0.282	0.281	0.282

Table 3.004c. Urinary excretion data table for patient 004.

Test samples	Time interval dt (hrs).	Urine vol. (mls).	Urine conc. μg/ml.	Amt. excreted (du)	Cum. Amt. excreted U.	Mid pt. time t.	Rate of excretion du/dt.	A.R.E U^{∞}-U.
T1	0 – 4	204	1.6134	329.134	329.134			1706.288
	4					2	82.2834	
T2	4 – 9	235	1.6975	398.913	728.046			1307.375
	5					6.5	99.7281	
T3	9 – 13	175	2.3950	419.125	1147.17			888.25
	4					11	104.781	
T4	13 – 17	100	3.2353	323.53	1470.70			564.72
	4					15	80.8825	
T5	17 – 21	200	1.9412	388.24	1858.94			176.48
	4					19	97.06	
T6	21 – 25	200	0.8824	176.48	2035.42			0.00
	4					23	44.12	

fe = U^{∞}/DOSE = 0.0136

3.004b. URINARY EXCRETION-TIME PLOTS FOR PATIENT 004.

Fig 3.004a. Calibration curve for patient 004. r^2=0.9909
m=0.2380, c=0.0720; Y = 0.2380X + 0.0720

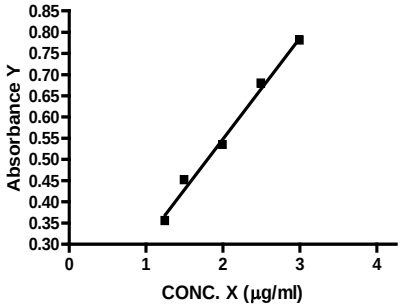


Fig 3.004b. A.R.E plot for patient 004.
r^2=0.8898, kel=0.1265, fe=0.0243

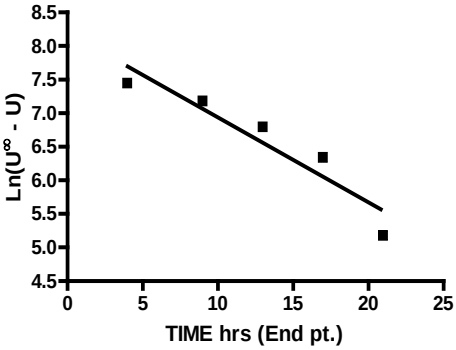


Fig 3.004c. Excretion rate plot for patient 004.

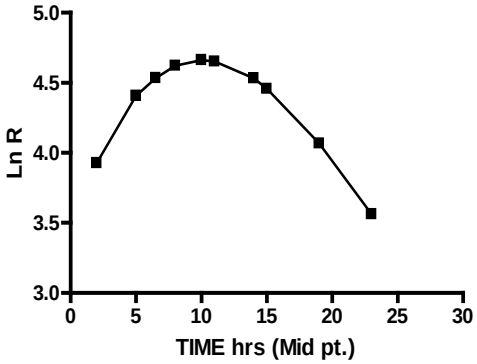


Fig 3.004d. Residuals plot for patient 004.
Residuals slope=ka=0.2600, $t_{1/2}$a=2.6654, (r^2=0.9781)
Terminal slope=kel=0.1080, $t_{1/2}$=6.4167, (r^2=0.9991)

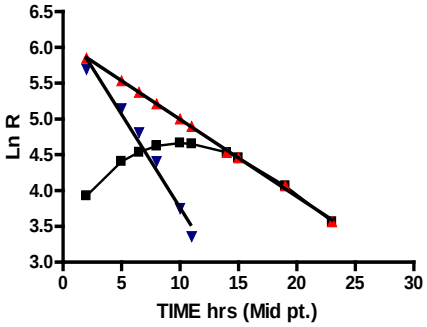


Table 3.004d. Residuals data table for patient 004.

Time	R	LnR	LnR^{last}	R^{last}	R^{last} - R	$Ln(R^{last} - R)$
2	50.7545	3.927	5.852	347.9295	297.175	5.6942
5	82.0230	4.407	5.533	252.9015	170.8785	5.1410
6.5	93.2235	4.535	5.373	215.5084	122.2849	4.8064
8	101.799	4.623	5.213	183.6442	81.8452	4.4048
10	105.954	4.663	5.000	148.4132	42.4597	3.7486
11	104.899	4.653	4.894	133.4865	28.5873	3.3530
14	92.9443	4.532	4.532			
15	86.2284	4.457	4.457			
19	58.3816	4.067	4.067			
23	35.2686	3.563	3.563			

3.005a. Data for patient 005

Table 3.005a. Control samples absorbance data table.

CONTROL SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
1.25	0.262	0.261	0.262
1.50	0.396	0.397	0.397
2.00	0.412	0.412	0.412
2.50	0.473	0.474	0.474
3.00	0.620	0.620	0.620

This data was used to draw the calibration curve, figure 3.005a.

Table 3.005b.Test samples absorbance data table.

TEST SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
T1: 0.0007	0.009	0.009	0.009
T2: 0.0579	0.016	0.017	0.017
T3: 0.4010	0.065	0.065	0.065
T4: 0.2652	0.045	0.046	0.046
T5 : 0.2009	0.037	0.037	0.037
T6: 0.2366	0.042	0.041	0.042

Table 3.005c. Urinary excretion data table for patient 005.

Test samples	Time interval dt (hrs).	Urine vol. (mls).	Urine conc. μg/ml.	Amt. excreted (du)	Cum. Amt. excreted U.	Mid pt. time t.	Rate of excretion du/dt.	A.R.E U^{∞}-U.
T1	0 – 4	178	0.0007	0.1246	0.1246			279.196
	4					2	0.0312	
T2	4 – 8	348	0.0579	20.1492	20.2738			259.047
	4					6	5.0373	
T3	8 – 12	87	0.4010	34.887	55.1608			224.16
	4					10	8.7218	
T4	12 – 16	334	0.2652	88.579	143.737			135.583
	4					14	22.144	
T5	16 – 20	450	0.2009	90.405	234.143			45.178
	4					18	22.601	
T6	20 – 24	140	0.2366	33.124	267.267			12.054
	4					22	8.281	
T7	24 – 28	100	0.1205	12.054	279.321			0.000
	4					26	3.0135	

$fe = U^{\infty}/DOSE = 0.0019$

3.005b. URINARY EXCRETION-TIME PLOTS FOR PATIENT 005.

Fig 3.005a. Calibration curve for patient 005; r^2=0.9026
m=0.1399; C=0.0089: Y=0.1399X + 0.0089

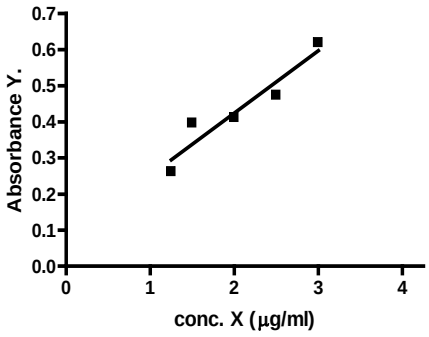


Fig 3.005b. A.R.E Plt for patient 005
r^2=0.8427; kel=0.1532; fe=0.0059

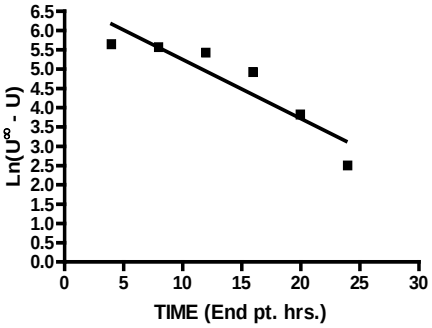


Fig 3.005c. Excretion rate plot for patient 005

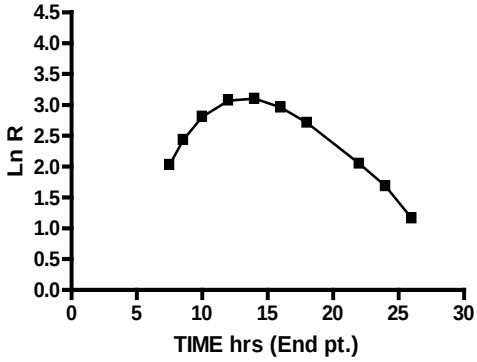


Fig 3.005d. Residual plot for patient 005;
Residual slope=ka=0.3923, $t_{1/2}$a=1.7665 , (r^2=0.9804)
Terminal slope=kel=0.1884, $t_{1/2}$=3.6784 , (r^2=0.9982)

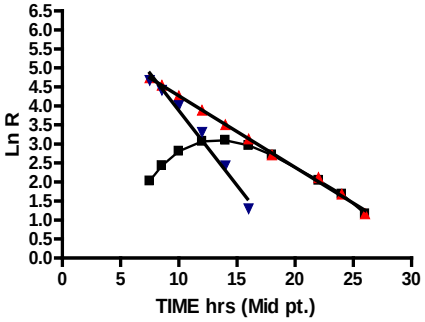


Table 3.005 d. Method of Residuals data table for patient 005.

TIME	R	LnR	Ln R^{last}	R^{Last}	$R^{last} - R$	Ln($R^{last} - R$)
7.5	7.5837	2.026	4.7418	114.6404	107.0567	4.6733
8.55	11.3702	2.431	4.5428	93.9535	82.5833	4.4138
10	16.5767	2.808	4.2680	71.3787	54.802	4.0037
12	21.5635	3.071	3.8890	48.8620	27.2985	3.3038
14	22.1536	3.098	3.5100	33.4483	11.2947	2.4243
16	19.2594	2.958	3.1310	22.8969	3.6375	1.2913
18	15.0143	2.709	2.709			
22	8.2813	2.114	2.114			
24	5.3709	1.681	1.681			
26	3.1899	1.160	1.160			

3.006a. Data for patient 006.

Table 3.006a. Control samples absorbance data table.

CONTROL SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
1.00	0.604	0.604	0.604
1.25	0.668	0.668	0.668
1.50	0.671	0.670	0.671
2.00	0.807	0.806	0.807
2.50	0.995	0.994	0.995
3.00	1.088	1.088	1.088

This data was used to draw the calibration curve, figure 3.006a.

Table 3.006b.Test samples absorbance data table.

TEST SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
T1; 0.4146	0.130	0.129	0.130
T2; 0.3034	0.098	0.098	0.098
T3; 5.8861	1.360	1.360	1.360
T4; 9.9786	2.280	2.280	2.280
T5; 0.8797	0.069	0.068	0.069
T6	-	-	-

Table 3.006c. Urinary excretion data table for patient 006.

Test samples	Time interval dt (hrs).	Urine vol. (mls).	Urine conc. μg/ml.	Amt. excreted (du)	Cum. Amt. excreted U.	Mid pt. time t.	Rate of excretion du/dt.	A.R.E U^{∞}-U.
T1	0 – 4	229	0.4146	94.9434	94.9434			1163.543
	4					2	23.736	
T2	4 – 8	408	0.3034	123.787	218.731			1039.756
	4					6	30.947	
T3	8 – 12	67	5.8861	394.369	613.099			645.3872
	4					10	98.592	
T4	12 – 16	28	9.9786	279.401	892.500			365.9864
	4					14	69.850	
T5	16 – 24	416	0.8797	365.986	1258.49			-
	8					20	45.748	

fe = U^{∞}/DOSE = 0.0084

3.006b. URINARY EXCRETION-TIME PLOTS FOR PATIENT 006.

Fig 3.006a ;Calibration curve for patient 006; r^2=0.9608. m=0.2248;c=0.0368; Y=0.2248X + 0.0368

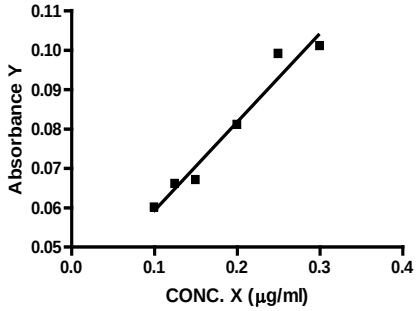


Fig 3.006b :A.R.E. plot for patient 006; r^2=0.9335;kel=0.0987;fe=0.0131

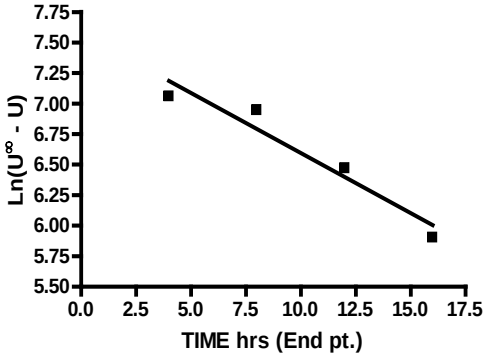


Fig 3.006c. Excretion rate plot for patient 006.

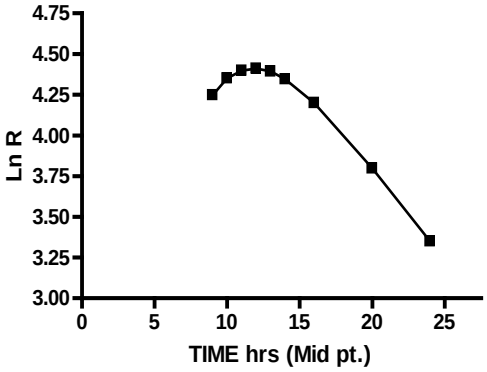


Fig 3.006d. Residual plot for patient 006.
Residual slope=ka=0.4917, $t_{1/2}$a=1.4094, (r^2=0.9591)
Terminal slope=kel=0.1062, $t_{1/2}$=6.5254, (r^2=0.9999)

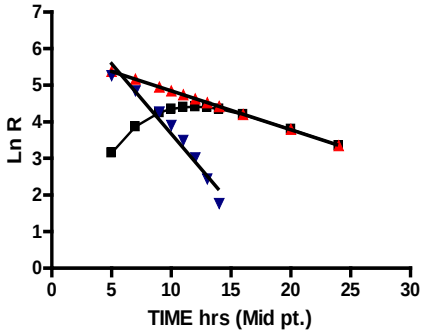


Table 3.006d. Residual plot data table for patient 006.

Time	R	Ln R	Ln R^{last}	R^{last}	R^{last} - R	Ln(R^{last} – R)
5	23.3127	3.149	5.3755	216.0479	192.7352	5.2613
7	47.7033	3.865	5.1629	174.6703	126.967	4.8439
9	69.8954	4.247	4.9503	141.2173	71.3219	4.2672
10	77.556	4.351	4.8440	126.9762	49.4202	3.9004
11	81.2881	4.398	4.7377	114.1713	32.8832	3.4930
12	82.3518	4.411	4.6316	102.6577	20.3059	3.0109
13	80.8827	4.393	4.5251	92.3052	11.4225	2.4356
14	77.1692	4.346	4.4188	82.9966	5.8274	1.7626
16	66.6863	4.200	4.2000			

20	44.5673	3.797	3.7970			
24	28.5027	3.350	3.3500			

3.007a. Data for patient 007

Table 3.007a. Control samples absorbance data table.

CONTROL SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
1.00			
1.25	0.323	0.322	0.323
1.50	0.364	0.363	0.364
2.00	0.543	0.543	0.543
2.50	0.653	0.653	0.653
3.00	0.697	0.696	0.697

This data was used to draw the calibration curve, figure 3.007a.

Table 3.007b.Test samples absorbance data table.

TEST SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
T1; 0.1578	0.082	0.081	0.082
T2; 0.1491	0.080	0.079	0.080
T3; 2.1718	0.544	0.544	0.544
T4; 1.2040	0.322	0.321	0.322
T5; 0.2319	0.099	0.098	0.099
T6; 0.0531	0.058	0.057	0.058
T7; 0.0023	0.046	0.045	0.046
T8 0.0968	0.068	0.068	0.068

Table 3.007c. Urinary excretion data table for patient 007.

Test samples	Time interval dt (hrs).	Urine vol. (mls).	Urine conc. μg/ml.	Amt. excreted (du)	Cum. Amt. excreted U.	Mid pt. time t.	Rate of excretion du/dt.	A.R.E U^{∞}-U.
T1	0 – 3	135	0.1578	21.303	21.303			194.74
	3.00					1.5	7.101	
T2	3 – 6	85	0.1491	12.6735	33.9765			182.07
	3.00					4.5	10.83	
T3	6 – 9	27	2.1718	58.6386	92.6151			123.43
	3.00					7.5	18.49	
T4	9-12	67	1.2040	80.668	173.283			42.76
	3.00					10.5	23.05	
T5	12-15	112	0.2319	25.9728	199.256			16.787
	3.00					13.5	14.19	
T6	15 - 18	277	0.0531	14.7087	213.965			2.078
	3.00					16.5	2.45	
T7	18 - 24	460	0.0023	1.058	215.023			1.020
	6.00					21.0	0.18	
T8	24 - 36	80	0.0128	1.020	216.043			-

	12.00					30.0	0.085	

fe = U^{∞}/DOSE = 0.0014

3.007b. URINARY EXCRETION-TIME PLOTS FOR PATIENT 007.

Fig 3.007a. Calibration curve for patient 007. r^2=0.9579
m=0.2294, c=0.0458; Y=0.2294X + 0.0458

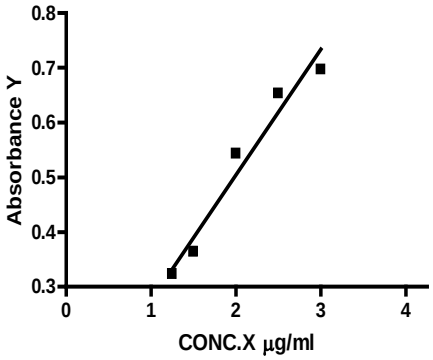


Fig 3.007b. A.R.E plot for patient 007.
r^2=0.9605; kel=0.2965; fe=0.0047

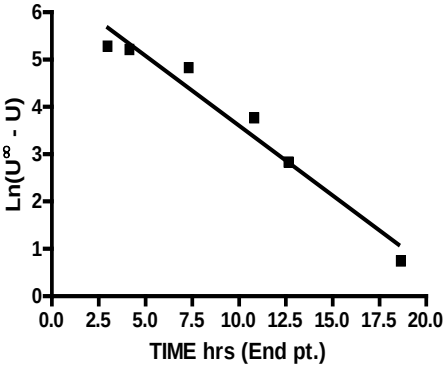


Fig 3.007c. Excretion rate plot for patient 007.

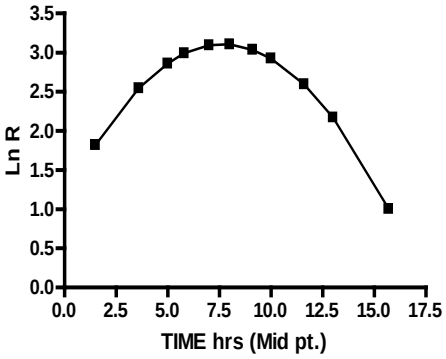


Fig 3.007d. Residual plot for patient 007;
Residual slope=ka=0.4471, $t_{1/2}$a=1.5500 , (r^2=0.9545)
Terminal slope=kel=0.2224, $t_{1/2}$=3.116 , (r^2=0.9982)

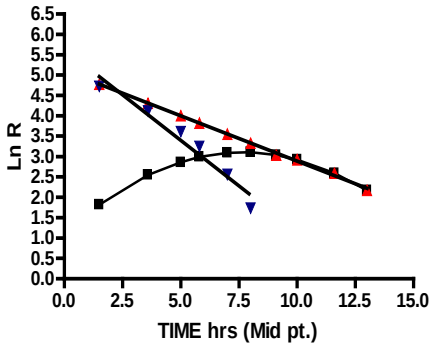


Table 3.007d. Residual data table for patient 007.

Time	R	Ln R	Ln R^{last}	R^{last}	R^{last} - R	Ln (R^{last}-R)
1.5	6.1595	1.818	4.7764	118.6763	112.517	4.7231
3.6	12.756	2.546	4.3094	74.3958	61.6398	4.1213
5.0	17.4615	2.860	3.9980	54.4891	37.0276	3.6117
5.8	19.8857	2.990	3.8200	45.6042	25.7185	3.2472
7.0	22.0211	3.092	3.5532	34.9249	12.9038	2.5575
8.0	22.3092	3.105	3.3308	27.9607	5.6515	1.7319
9.1	20.7802	3.034	3.034			
10	18.6342	2.925	2.925			
11.6	13.3698	2.593	2.593			

13.0	8.7583	2.170	2.170			

3.008a. Data for patient 008

Table 3.008a. Control samples absorbance data table.

CONTROL SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
1.00			
1.25	0.326	0.327	0.327
1.50	0.456	0.455	0.456
2.00	0.551	0.551	0.551
2.50	0.664	0.665	0.665
3.00	0.781	0.780	0.781

This data was used to draw the calibration curve, figure 3.008a.

Table 3.008b.Test samples absorbance data table.

TEST SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
T1; 8.1	2.030	2.030	2.030
T2; 11.7	2.920	2.910	2.920
T3; 5.7	1.448	1.449	1.449
T4; 0.7	0.120	0.120	0.120
T5; 0.4	0.091	0.090	0.091
T6; 1.0	0.149	0.148	0.149

Table 3.008c. Urinary excretion data table for patient 008.

Test samples	Time interval dt (hrs).	Urine vol. (mls).	Urine conc. μg/ml.	Amt. excreted (du)	Cum. Amt. excreted U.	Mid pt. time t.	Rate of excretion du/dt.	A.R.E U^{∞}-U.
T1	0 – 4	50	8.1	405.0	405.0			1585.8
	4					2	101.25	
T2	4 – 8	48	11.7	561.6	966.6			1024.2
	4					6	140.4	
T3	8 – 12	74	5.7	421.8	1388.4			602.4
	4					10	105.45	
T4	12 – 16	500	0.7	350.0	1738.4			252.4
	4					14	87.5	
T5	16 – 20	261	0.4	104.4	1842.8			148.0
	4					18	56.1	
T6	20 – 24	148	1.0	148.0	1990.8			-
	4					22	37.0	
T7	24 – 28							
	4					26		

fe = U^{∞}/DOSE = 0.0033

3.008b. URINARY EXCRETION-TIME PLOTS FOR PATIENT 008.

Fig 3.008a. Calibration curve for patient 008. r^2=0.9807 m=0.2445, c=0.0548; Y=0.2445X + 0.0548

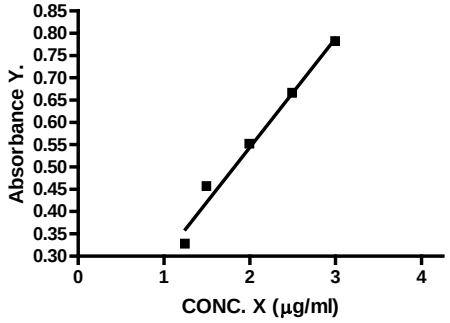


Fig 3.008b: A.R.E. plot for patient 008; r^2=0.9867; kel=0.1536, fe=0.0054

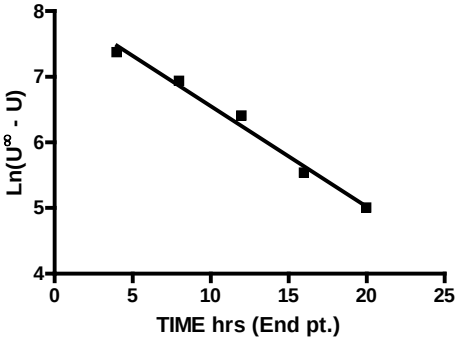


Fig 3.008c. Excretion rate plot for patient 008.

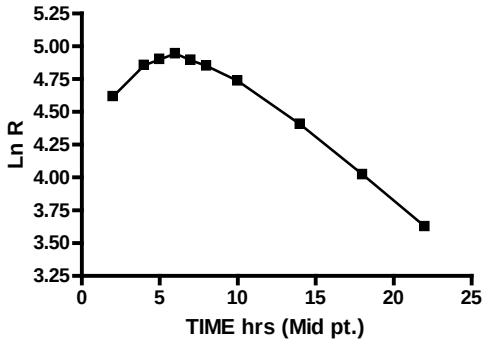


Fig 3.008d. Residual plot for patient 008.
Residual slope=ka=0.4200, $t_{1/2}$a=1.6500, (r^2=0.9908)
Terminal slope=kel=0.1528, $t_{1/2}$=4.5353, (r^2=0.9996)

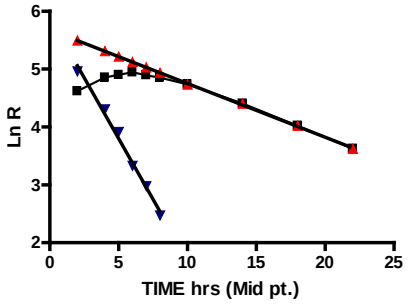


Table 3.008d. Residuals data table for patient 008.

Time	R	Ln R	Ln Rlast	Rlast	Rlast - R	Ln (Rlast-R)
2	101.291	4.618	5.4964	243.8126	142.5216	4.9595
4	128.509	4.856	5.3108	202.5122	74.0032	4.3041
5	134.424	4.901	5.2180	184.5647	50.1407	3.9148
6	140.330	4.944	5.1252	168.2078	27.8778	3.3278
7	133.620	4.895	5.0324	153.3005	19.6805	2.9796
8	127.868	4.851	4.9396	139.7144	11.8464	2.4720
10	113.977	4.736	4.736			
14	81.859	4.405	4.405			
18	55.869	4.023	4.023			
22	37.562	3.626	3.626			

3.009a. Data for patient 009

Table 3.009a. Control samples absorbance data table.

CONTROL SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
1.00			
1.25	0.236	0.235	0.236
1.50	0.295	0.295	0.295
2.00	0.331	0.332	0.332
2.50	0.392	0.392	0.392
3.00	0.499	0.498	0.499

This data was used to draw the calibration curve, figure 3.009a.

Table 3.009b.Test samples absorbance data table.

TEST SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
T1; 3.2029	0.510	0.510	0.510
T2; 1.1594	0.228	0.227	0.228
T3; 0.3696	0.119	0.118	0.119
T4; 0.3623	0.118	0.117	0.118
T5; 0.3551	0.117	0.116	0.117
T6; 0.1816	0.093	0.092	0.093

Table 3.009c. Urinary excretion data table for patient 009.

Test samples	Time interval dt (hrs).	Urine vol. (mls).	Urine conc. μg/ml.	Amt. excreted (du)	Cum. Amt. excreted U.	Mid pt. time t.	Rate of excretion du/dt.	A.R.E U^{∞}-U.
T1	0 – 3	30	3.2029	96.0870	96.0870			176.2204
	3					1.5	33.3635	
T2	3 - 6	76	1.1594	88.1144	184.201			88.1060
	3					4.5	44.1572	
T3	6 - 9	75	0.3696	27.7200	211.921			60.3860
	3					7.5	10.2667	
T4	9 - 12	50	0.3623	18.1150	230.036			42.2710
	3					10.5	6.5873	
T5	12 - 15	50	0.3551	17.7550	247.791			24.5160
	3					13.5	7.8911	
T6	15 -21	135	0.1816	24.5160	272.307			-
	6					18.0	3.304	

fe = U^{∞}/DOSE = 0.0018

3.009b. URINARY EXCRETION-TIME PLOTS FOR PATIENT 009

Fig 3.009a. Calibration curve for patient 009. r^2=0.9826; m=0.1148, c=0.1055; Y=0.1148X + 0.1055

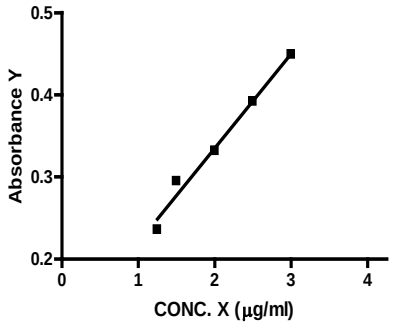


Fig 3.009b. A.R.E plot for patient 009. r^2=0.9704; kel=0.1866, fe=0.0017

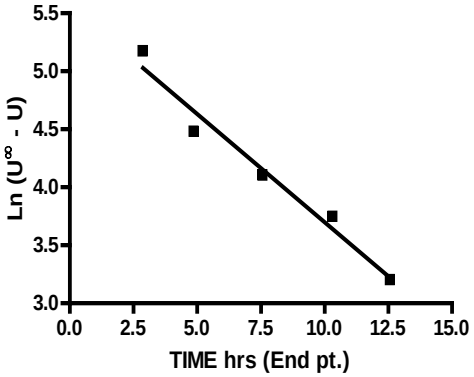


Fig 3.009c. Excretion rate plot for patient 009.

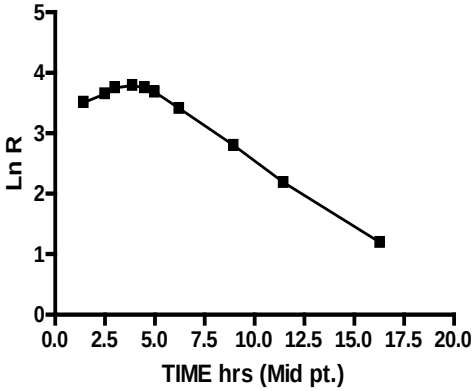


Fig 3.009d. Residuals plot for patient 009. Residuals slope=ka=0.8898, $t_{1/2}$a=0.7788, (r^2=0.9495) Terminal slope=kel=0.2212, $t_{1/2}$=3.1329, (r^2=0.9996)

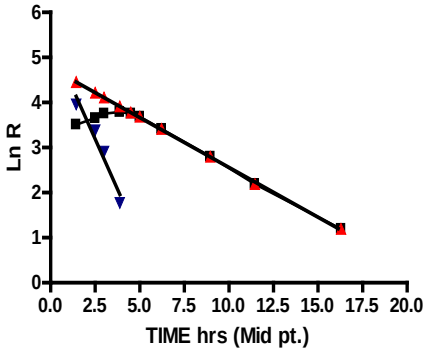


Table 3.009d. Residuals data table for patient 009

Time	R	Ln R	Ln (R^{last})	R^{last}	R^{last} - R	Ln (R^{last}-R)
1.44	33.4148	3.509	4.4528	85.8670	52.4522	3.9599
2.50	38.4747	3.650	4.2185	67.9315	29.4568	3.3829
3.00	42.5211	3.750	4.1080	60.8249	18.3038	2.9071
3.88	44.2122	3.789	3.9135	50.0739	5.8617	1.7684
4.50	42.5211	3.750	3.7765	43.663	1.1419	0.1327
5.00	39.6464	3.680	3.680			
6.23	30.2955	3.411	3.411			
8.96	16.4446	2.800	2.800			
11.46	8.8995	2.186	2.186			

16.29	3.3003	1.194	1.194			

3.010a. Data for patient 010

Table 3.010a. Control samples absorbance data table.

CONTROL SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
1.00			
1.25	0.218	0.217	0.218
1.50	0.275	0.275	0.275
2.00	0.320	0.321	0.321
2.50	0.433	0.433	0.433
3.00	0.499	0.498	0.499

This data was used to draw the calibration curve, figure 3.010a.

Table 3.010b.Test samples absorbance data table.

TEST SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
T1; 0.0815	0.035	0.034	0.035
T2; 0.1817	0.051	0.051	0.051
T3; 0.1190	0.040	0.041	0.041
T4; 0.0689	0.033	0.032	0.033
T5; 0.2068	0.055	0.055	0.055
T6; 0.0501	0.029	0.030	0.030
T7; 0.0063	0.023	0.022	0.023

Table 3.010c. Urinary excretion data table for patient 010.

Test samples	Time interval dt (hrs).	Urine vol. (mls).	Urine conc. μg/ml.	Amt. excreted (du)	Cum. Amt. excreted U.	Mid pt. time t.	Rate of excretion du/dt.	A.R.E U^{∞}-U.
T1	0 – 3	195	0.0815	15.8925	15.8925			77.9004
	3					1.5	6.282	
T2	3 - 6	62	0.1817	11.2654	27.1579			66.6350
	3					4.5	14.08	
T3	6 - 9	125	0.1190	14.875	42.0329			51.7600
	3					7.5	11.998	
T4	9 - 12	240	0.0689	16.536	58.5689			35.2240
	3					10.5	9.134	
T5	12 - 15	100	0.2068	20.68	79.2489			14.5440
	3					13.5	7.863	
T6	15 - 18	240	0.0501	12.024	91.2729			2.5200
	3					16.5	6.680	
T7	18 - 24	400	0.0063	2.5200	93.7929			-
	6					21.0	1.280	

fe = U^{∞}/DOSE = 0.0012

3.010b. URINARY EXCRETION-TIME PLOTS FOR PATIENT 010

Fig 3.010a. Calibration curve for patient 010; r^2=0.9858, m=0.1596, c=0.0220; Y= 0.1596X + 0.0220

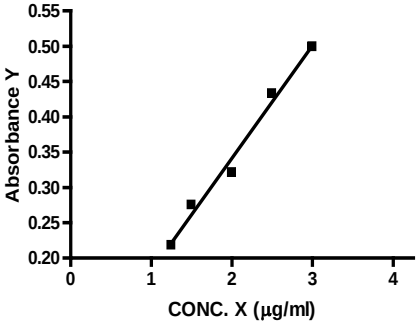


Fig 3.010b. A.R.E. plot for patient 010, r^2=0.8527, kel=0.2406, fe=0.0012

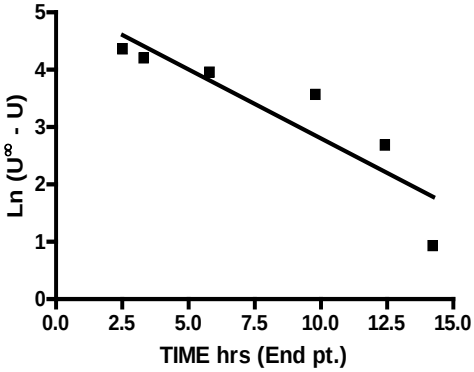


Fig 3.010c. Excretion rate plot for patient 010;

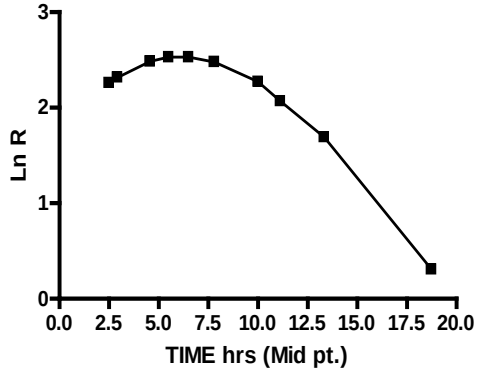


Fig 3.010d. Residuals plot for patient 010.
Residual slope=ka=0.4118, $t_{1/2}$a=1.6829, (r^2=0.9894)
Terminal slope=kel=0.2273, $t_{1/2}$=3.0488, (r^2=0.9984)

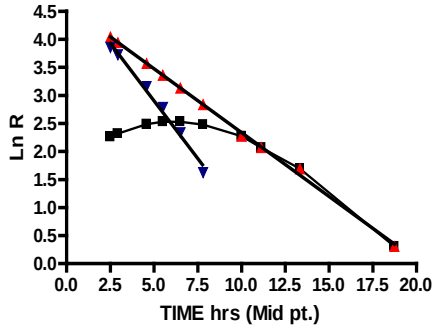


Table 3.010d. Residuals data table for patient 010

Time	R	Ln R	Ln R^{last}	R^{last}	R^{last}-R	Ln (R^{last}-R)
1.27	5.5124	1.707	4.3183	75.0609	69.5485	4.2420
2.5	9.5634	2.258	4.0388	56.7582	47.1942	3.8543
2.93	10.1351	2.316	3.941	51.4700	41.3349	3.7217
4.57	11.9891	2.484	3.5682	35.4527	23.4636	3.1555
5.5	12.5284	2.528	3.3569	28.7001	16.1717	2.7833
6.5	12.541	2.529	3.130	22.874	10.333	2.3353
7.81	11.9174	2.478	2.8318	16.976	5.0586	1.6211
10	9.6891	2.271	2.271			
11.13	7.909	2.068	2.068			
13.34	5.4194	1.69	1.69			

18.74	1.3566	0.305	0.305			

3.011a. Data for patient 011

Table 3.011a. Control samples absorbance data table.

CONTROL SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
1.00	0.362	0.362	0.362
1.25	0.387	0.386	0.387
1.50	0.393	0.394	0.394
2.00	0.486	0.485	0.486
2.50	0.514	0.513	0.514
3.00	0.674	0.675	0.675

This data was used to draw the calibration curve, figure 3.011a; Y = 0.1465X + 0.1949

Table 3.011b.Test samples absorbance data table.

TEST SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
T1; 1.6667	0.045	0.045	0.045
T2; 19.4	0.311	0.310	0.311
T3; 19.2667	0.309	0.309	0.309
T4; 0.7333	0.031	0.031	0.031
T5; 0.8667	0.033	0.033	0.033
T6; 24.1333	0.382	0.382	0.382
T7; 16.8	0.272	0.273	0.273

Table 3.011c. Urinary excretion data table for patient 011.

Test samples	Time interval dt (hrs).	Urine vol. (mls).	Urine conc. μg/ml.	Amt. excreted (du)	Cum. Amt. excreted U.	Mid pt. time t.	Rate of excretion du/dt.	A.R.E U^{∞}-U.
T1	0 – 4	181	1.6667	301.673	301.673			11321.41
	4					2	150.84	
T2	4 – 8	120	19.400	2328.00	2629.67			8993.41
	4					6	253.9	
T3	8 - 12	188	19.267	3622.14	6251.81			5371.27
	4					10	1811.07	
T4	12 - 16	343	0.7333	251.522	6503.34			5119.748
	4					14	58.09	
T5	16 - 20	440	0.8667	381.348	6884.68			4738.400
	4					18	76.27	
T6	20 - 24	78	24.133	1882.39	8767.08			2856.000
	4					22	564.65	
T7	24 - 28	170	16.8	2856.00	11623.1			-
	4					26	317.333	

fe = U^{∞}/DOSE = 0.0076

3.011b. URINARY EXCRETION-TIME PLOTS FOR PATIENT 011

Fig 3.011a; Calibration curve for patient 011, r^2=0.9299
m=0.1465,c=0.1949; Y=0.1465X + 0.1949

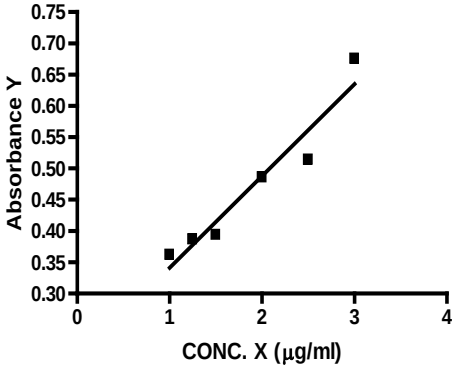


Fig 3.011b: A.R.E plot for patient 011.
r^2=0.9192; kel=0.0853, fe=0.0758

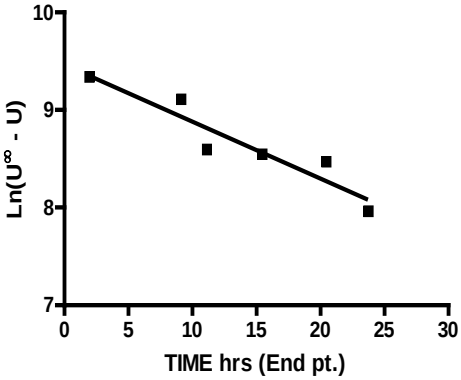


Fig 3.011c; Excretion rate plot for patient 011.

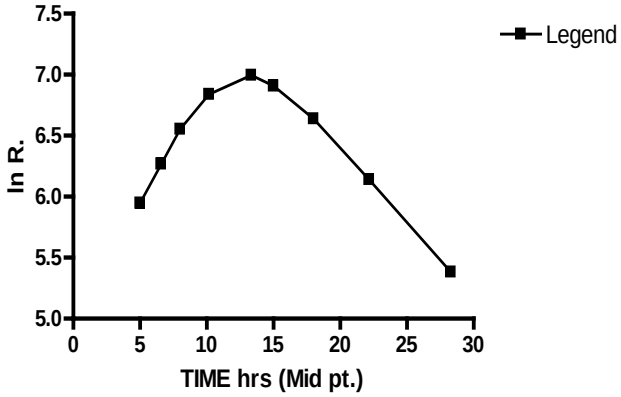


Fig 3.011d; Residual plots for patient 011.
Residual slope=ka=0.3381, $t_{1/2}$a=2.0497, (r^2=0.9496)
Terminal slope=kel=0.1162, $t_{1/2}$=5.9639, (r^2=0.9993)

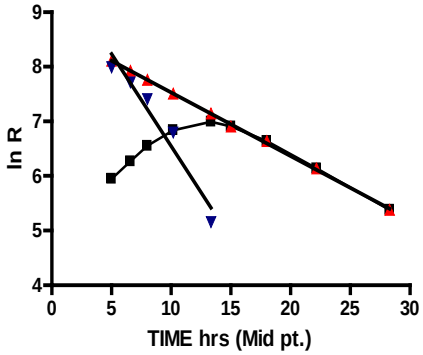


Table 3.011d. Residuals data table for patient 011.

Time	R	LnR	Ln R^{last}	R^{last}	R^{last} - R	Ln(R^{last} – R)
5.00	382.221	5.946	8.1110	3330.907	2948.686	7.9891
6.59	526.894	6.267	7.9262	2768.885	2241.991	7.7151
8.00	702.047	6.554	7.7624	2350.539	1648.492	7.4076
10.17	933.555	6.839	7.5102	1826.579	893.024	6.7946
13.34	1090.07	6.994	7.1419	1263.827	173.757	5.1577
15.00	1000.24	6.908	6.908			
18.00	764.33	6.639	6.639			
22.17	464.518	6.141	6.141			
28.30	217.239	5.381	5.381			

3.012a. Data for patient 012

Table 3.012a. Control samples absorbance data table.

CONTROL SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
1.00	0.337	0.336	0.337
1.25	0.454	0.455	0.455
1.50	0.473	0.472	0.473
2.00	0.549	0.548	0.549
2.50	0.711	0.710	0.711
3.00	0.751	0.750	0.751

This data was used to draw the calibration curve, figure 3.012a.

Table 3.012b.Test samples absorbance data table.

TEST SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
T1; 1.5195	0.474	0.473	0.474
T2; 1.5491	0.480	0.479	0.480
T3; 2.8726	0.748	0.747	0.748
T4; 3.7516	0.926	0.926	0.926
T5; 1.6232	0.495	0.494	0.495
T6; 0.8232	0.333	0.332	0.333

Table 3.012c. Urinary excretion data table for patient 012.

Test samples	Time interval dt (hrs).	Urine vol. (mls).	Urine conc. μg/ml.	Amt. excreted (du)	Cum. Amt. excreted U.	Mid pt. time t.	Rate of excretion du/dt.	A.R.E U^{∞}-U.
T1	0 – 4	204	1.5195	309.978	309.978			1558.561
	4					2	77.4945	
T2	4 – 9	235	1.5491	364.039	674.017			1194.522
	5					6.5	91.018	
T3	9 – 13	175	2.8726	502.705	1176.72			691.817
	4					11	125.6763	
T4	13 – 17	103	3.7516	387.58	1364.30			504.237
	4					15	96.895	
T5	17 – 21	194	1.6232	314.901	1679.20			189.336
	4					19	78.7251	
T6	21 – 25	230	0.8232	189.336	1868.54			-
	4					23	47.334	

$fe = U^{\infty}/DOSE = 0.0125$

3.012b. URINARY EXCRETION-TIME PLOTS FOR PATIENT 012

Fig 3.012a. Calibration curve for patient 012; r^2=0.9622
m=0.2025, c=0.1663; Y=0.2025X + 0.1663

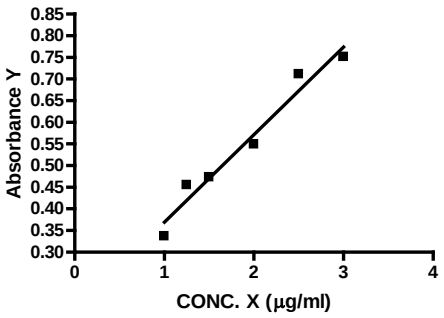


Fig 3.012b. A.R.E. plot for patient 012.
r^2=0.9622, kel=0.1198, fe=0.0203

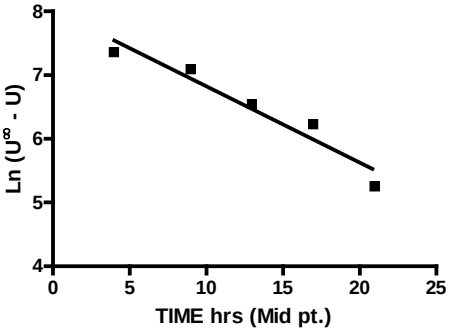


Fig 3.012c. Excretion rate plot for patient 012.

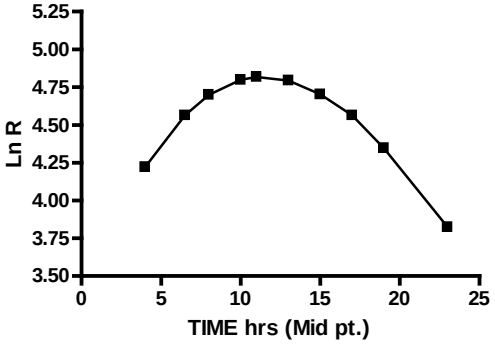


Fig 3.012d. Residuals plot for patient 012.
Residuals slope=ka=0.2580, $t_{1/2}$a=2.6860, (r^2=0.9650)
Terminal slope=kel=0.1119, $t_{1/2}$=6.1930, (r^2=0.9988)

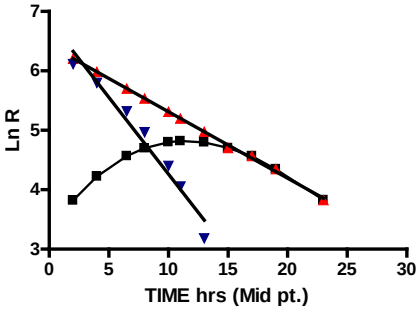


Table 3.012d. Residuals data table for patient 012

Time	R	Ln R	Ln R^{last}	R^{last}	R^{last} - R	Ln(R^{last} – R)
2	45.4676	3.817	6.2062	495.8136	450.346	6.1100
4	68.1016	4.221	5.9824	396.3906	328.289	5.7939
6.5	95.9666	4.564	5.7027	299.6754	203.7088	5.3167
8	109.947	4.700	5.5348	253.3571	143.4099	4.9657
10	121.268	4.798	5.3110	202.5527	81.2851	4.3980
11	123.594	4.817	5.1991	181.1092	57.5154	4.0521
13	120.784	4.794	4.9753	144.7923	24.0088	3.1784
15	110.167	4.702	4.702			
17	95.9666	4.564	4.564			
19	77.1692	4.346	4.346			
23	45.8328	3.825	3.825			

3.013 a. Data for patient 013

Table 3.013a. Control samples absorbance data table.

CONTROL SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
1.25	0.234	0.234	0.234
1.50	0.296	0.297	0.297
2.00	0.305	0.305	0.305
2.50	0.338	0.339	0.339
3.00	0.418	0.417	0.418

This data was used to draw the calibration curve, figure 3.013a.

Table 3.013b.Test samples absorbance data table.

TEST SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
T1; 0.2449	0.117	0.118	0.118
T2; 3.2381	0.338	0.337	0.338
T3; 8.7755	0.745	0.744	0.745
T4; 1.7687	0.230	0.229	0.230
T5; 0.1088	0.108	0.108	0.108
T6	-	-	-

Table 3.013c. Urinary excretion data table for patient 013.

Test samples	Time interval dt (hrs).	Urine vol. (mls).	Urine conc. μg/ml.	Amt. excreted (du)	Cum. Amt. excreted U.	Mid pt. time t.	Rate of excretion du/dt.	A.R.E U^{∞}- U.
T1	0 – 4	200	0.2449	48.9800	48.9800			1957.6241
	4					2	12.2450	
T2	4 – 8	275	3.2381	890.4775	939.4575			1067.1466
	4					6	222.62	
T3	8 – 12	92	8.7755	807.346	1746.8035			259.8006
	4					10	201.835	
T4	12 – 16	136	1.7687	240.543	1987.3465			19.2576
	4					14	60.136	
T5	16 – 20	177	0.1088	19.2576	2006.6041			-
	4					18	4.815	
T6	20 – 24	260						
	4					22		

$fe = U^{\infty}/DOSE = 0.0134$

3.013b. URINARY EXCRETION-TIME PLOTS FOR PATIENT 013

Fig 3.013 a. Calibration curve for patient 013. r^2=0.9107 m=0.0735, c=0.1000; Y=0.0735X + 0.1000

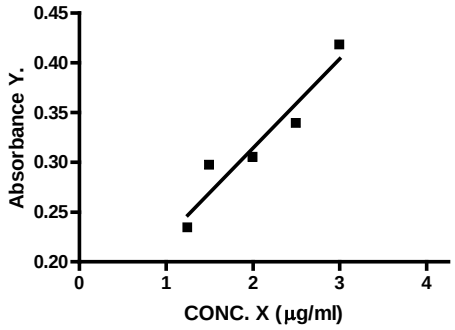


Fig 3.013 b. A.R.E plot for patient 013. r^2=0.9209; kel=0.1819; fe=0.0972

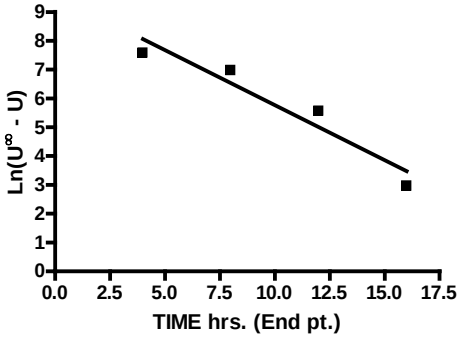


Fig 3.013c. Excretion rate plot for patient 013.

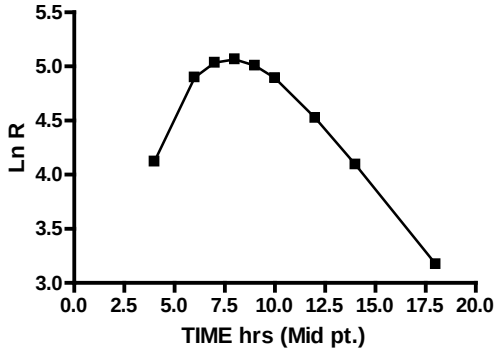


Fig 3.013d. Residuals plot for patient 013. Residuals slope=ka=0.5893, $t_{1/2}$a=1.1759, (r^2=0.9694) Terminal slope=kel=0.2168, $t_{1/2}$=3.1965 (r^2=0.9995)

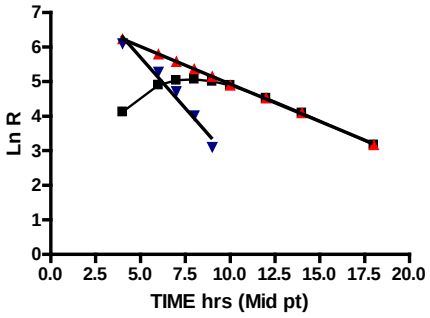


Table 3.013d. Method of residuals data table for patient 013.

Time	R	Ln R	Ln R^{last}	R^{last}	R^{last} – R	Ln (R^{last} – R)
2	11.9413	2.480	6.6636	783.3659	771.4247	6.6482
4	61.6208	4.121	6.2302	507.8570	446.2362	6.1008
6	134.156	4.899	5.7968	329.2443	195.0883	5.2735
7	153.7	5.035	5.5801	265.0981	111.3981	4.7131
8	158.38	5.065	5.3634	213.4494	55.0694	4.0086
9	149.605	5.008	5.1467	171.8634	22.2584	3.1027
10	133.353	4.893	4.893			
12	92.2037	4.524	4.524			
14	59.9793	4.094	4.094			

18	23.879	3.173	3.173			

3.014a. Data for patient 014

Table 3.014a. Control samples absorbance data table.

CONTROL SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
1.00			
1.25	0.517	0.518	0.518
1.50	0.718	0.717	0.718
2.00	0.945	0.944	0.945
2.50	1.124	1.125	1.125
3.00	1.161	1.162	1.162

This data was used to draw the calibration curve, figure 3.014a.

Table 3.014b.Test samples absorbance data table.

TEST SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
T1; 0.8107	0.438	0.438	0.438
T2; 2.1872	0.944	0.943	0.944
T3; 1.7791	0.794	0.793	0.794
T4; 1.975	0.866	0.866	0.866
T5; 1.9396	0.853	0.852	0.853
T6; 0.3727	0.277	0.276	0.277

Table 3.014c. Urinary excretion data table for patient 014.

Test samples	Time interval dt (hrs).	Urine vol. (mls).	Urine conc. μg/ml.	Amt. excreted (du)	Cum. Amt. excreted U.	Mid pt. time t.	Rate of excretion du/dt.	A.R.E U^{∞}-U.
T1	0 – 4	130	0.8107	105.391	105.391			972.6955
	4					2	25.1303	
T2	4 – 8	125	2.1872	273.400	378.791			699.2955
	4					6	52.3562	
T3	8 – 12	85	1.7791	151.224	530.015			548.072
	4					10	37.8059	
T4	12 – 18	100	1.9750	197.500	727.515			350.572
	6					15	32.9167	
T5	18 – 24	150	1.9396	290.940	1018.45			59.632
	6					21	48.490	
T6	24 – 30	160	0.3727	59.632	1078.09			-
	6					27	9.9387	

fe = U^{∞}/DOSE = 0.0072

3.014b URINARY EXCRETION-TIME PLOTS FOR PATIENT 014

Figure 3.014a. Calibration curve for patient 014, r^2=0.9230 m=0.3676, c=0.1400; Y=0.3676X + 0.1400

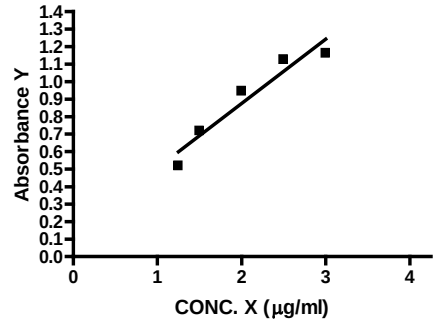


Figure 3.014b. A.R.E. plot for patient 014; r^2=0.9136; kel=0.1291; fe=0.0033

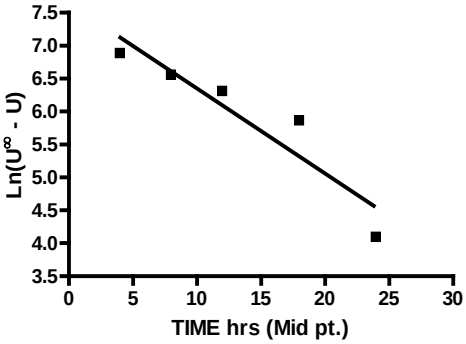


Fig 3.014c. Excretion rate plot for patient 014

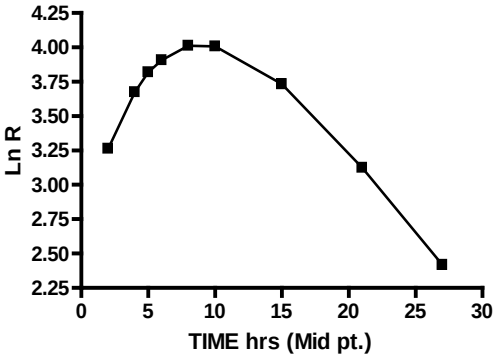


Fig 3.014d. Residuals plot for patient 014. Residual slope=ka=0.2651, $t_{1/2}$a=2.6141, (r^2=0.9831) Terminal slope=kel=0.1096, $t_{1/2}$=6.323, (r^2=0.9998)

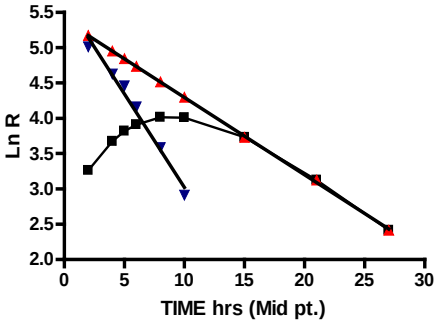


Table 3.014d. Method of residuals data table for patient 014.

Time	R	Ln R	Ln R^{last}	R^{last}	R^{last} - R	Ln (R^{last} – R).
2	26.1017	3.262	5.1728	176.4081	150.3064	5.0127
4	39.4092	3.674	4.9536	141.6841	102.2749	4.6277
5	45.6042	3.820	4.844	132.1582	86.554	4.4608
6	49.7993	3.908	4.7344	113.7952	63.9959	4.1588
8	55.3126	4.013	4.5152	91.3958	36.0832	3.5858
10	55.0367	4.008	4.296	73.4056	18.3689	2.9107
15	41.7625	3.732	3.732			
21	22.7371	3.124	3.124			
27	11.2122	2.417	2.417			

3.015a. Data for patient 015

Table 3.015a. Control samples absorbance data table.

CONTROL SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
1.00	0.375	0.374	0.375
1.25	0.389	0.388	0.389
1.50	0.411	0.411	0.411
2.00	0.524	0.523	0.524
2.50	0.554	0.553	0.554
3.00	0.700	0.699	0.700

This data was used to draw the calibration curve, figure 3.015a.

Table 3.015b.Test samples absorbance data table.

TEST SAMPLE CONC. Xμg/ml.	ABSORBANCE Y1.	ABSORBANCE Y2.	AVERAGE ABSORBANCE Y.
T1; 1.9813	0.051	0.050	0.051
T2; 17.6063	0.301	0.301	0.301
T3; 1.1688	0.038	0.038	0.038
T4; 1.6063	0.045	0.044	0.045
T5; 0.0438	0.020	0.020	0.020
T6	-	-	-

Table 3.015c. Urinary excretion data table for patient 015.

Test samples	Time interval dt (hrs).	Urine vol. (mls).	Urine conc. μg/ml.	Amt. excreted (du)	Cum. Amt. excreted U.	Mid pt. time t.	Rate of excretion du/dt.	A.R.E U^{∞}-U.
T1	0 – 4	181	1.9813	358.615	358.615			2902.723
	4					2	179.308	
T2	4 - 8	120	17.606	2112.76	2471.37			789.967
	4					6	294.666	
T3	8 - 12	188	1.1688	219.734	2691.11			570.233
	4					10	109.867	
T4	12 - 16	343	1.6063	550.961	3242.07			19.272
	4					14	127.243	

T5	16 - 24	440	0.0438	19.272	3261.34			-
	8					20	3.8544	

fe =U^{∞}/DOSE = 0.0069

3.015b. URINARY EXCRETION-TIME PLOTS FOR PATIENT 015.

Fig 3.015a. Calibration curve for patient 015.
r^2=0.9578, m=0.1596, c=0.1928; Y=0.1596X + 0.1928

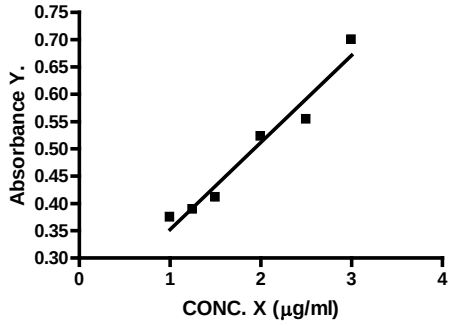


Fig 3.015b: A.R.E plot for patient 015.
r^2=0.8192, kel=0.3436, fe=0.0069

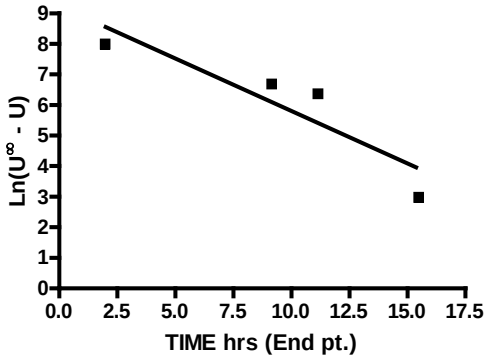


Fig 3.015c. Excretion rate plot for patient 015.

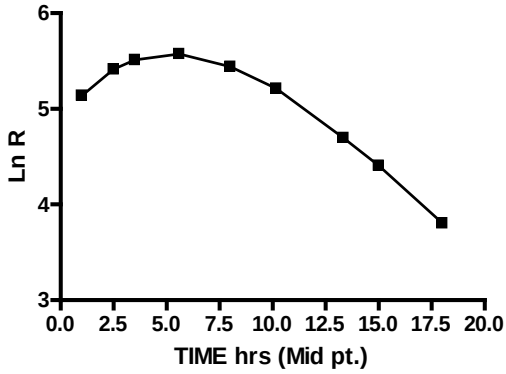


Fig 3.015d. Residuals plot for patient 015.
Residual slope=ka=0.4059, $t_{1/2}$a=1.7073, (r^2=0.9861)
Terminal slope=kel=0.1794, $t_{1/2}$=3.8629, (r^2=0.9996)

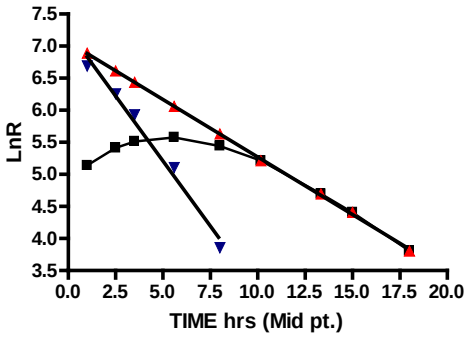


Table 3.015b. Residuals data table for patient 015.

Time	R	LnR	LnR^{last}	R^{last}	R^{last} - R	$Ln(R^{last} – R)$
1.00	170.545	5.139	6.8819	974.4761	803.9311	6.6895
2.50	224.303	5.413	6.613	744.7138	520.4108	6.2546
3.50	247.398	5.511	6.4338	622.5351	375.1371	5.9273
5.59	263.486	5.574	6.0593	428.0757	164.5897	5.1035

8.00	230.673	5.441	5.6274	277.9385	47.2655	3.8558
10.17	183.094	5.210	5.210			
13.34	109.508	4.696	4.696			
15.00	81.859	4.405	4.405			
18.00	44.880	3.804	3.804			

3.2 SUMMARY OF RESULTS.

Table 3.2 SUMMARY OF PHARMACOKINETICS OF ORAL AMODIAQUINE.

PATIENT'S CODE	fe	kel	$t_{1/2}$	ke	km	ka	$t_{1/2a}$
001	0.0066	0.1041	6.6571	0.0007	0.1081	0.6312	1.0979
002	0.0024	0.1247	5.5573	0.0003	0.1244	0.5411	1.2807
003	0.0044	0.1411	4.9114	0.0006	0.1405	0.4111	1.6857
004	0.0136	0.1080	6.4167	0.0015	0.1065	0.2600	2.6654
005	0.0019	0.1884	3.6784	0.0004	0.1880	0.3923	1.7665
006	0.0084	0.1062	6.5254	0.0009	0.1053	0.4917	1.4094
007	0.0014	0.2224	3.1160	0.0003	0.2221	0.4471	1.5500
008	0.0033	0.1528	4.5353	0.0005	0.1523	0.4200	1.6500
009	0.0018	0.2212	3.1329	0.0004	0.2208	0.8898	0.7788
010	0.0012	0.2273	3.0488	0.0003	0.2270	0.4118	1.6829
011	0.0076	0.1162	5.9639	0.0009	0.1153	0.3381	2.0497
012	0.0125	0.1119	6.1930	0.0014	0.1105	0.2580	2.6860
013	0.0134	0.2168	3.1965	0.0029	0.2139	0.5893	1.1759
014	0.0033	0.1096	6.3230	0.0004	0.1092	0.2651	2.6141
015	0.0069	0.1794	3.8629	0.0012	0.1782	0.4059	1.7073
MEAN (M)	0.0059	0.1553	4.8746	0.0008	0.1548	0.4502	1.7200
STDEV	0.0044	0.0488	1.4297	0.0006	0.0485	0.1657	0.5557
SEM	0.0011	0.0126	0.3691	0.0002	0.0125	0.0428	0.1435
95% CI	M+/-0.0024	M+/-0.027	M+/-0.7901	M+/-0.0004	M+/-0.0268	M+/-0.0916	M+/-0.3071

STDEV, is the standard deviation of the mean; SEM, is the standard error of the mean;

95% CI, is the 95% confidence interval levels or limits.

Table 3.3. 95% CI PK Values of Amodiaquine.

Parameter	Lower Limit	Upper Limit.
fe	0.0035	0.0083
kel	0.1283	0.1823
$t(_{1/2})$	4.0845	5.6647
ke	0.0004	0.0012
km	0.128	0.1816
ka	0.3586	0.5418

$t(_{1/2})a$	1.4129	2.0271

From literature, (Krishna et al., 1990) the pharmacokinetic parameter values of orally administered amodiaquine observed in seven healthy Caucasian adults were;

$t_{½}$; (5.2 +/- 1.7 hrs; n=7); range 1.0 – 9.4 hrs
kel; mean 0.13 hr^{-1}, (range 0.07 to 1.44 hr^{-1}.)

CL; mean 5.5, (range 1.6 – 17.3) L hr^{-1} kg^{-1}

V; mean 38.3 (range 3.7 – 127.9) L $kg^{-1.}$

From another source, P. A. Winstanley et al. 1990, the pharmacokinetic parameter values of amodiaquine following oral administration in fourteen (14) Zambian adults (i.e. East Africans) with uncomplicated malaria were observed as;

t ½ ; (3.7 +/- 0.35 hrs; n = 14); range 2.9 – 4.5 hrs

kel; mean 0.19 hr^{-1}; range 0.15 - 0.24 hr^{-1}.

3.3. Statistical analyses on Pharmacokinetic data of oral amodiaquine.

The pharmacokinetics data above, table 3.2, was further subjected to various statistical tests or analyses. (www.biology.ed.uk/statistics , {accessed 10 February 2009}).

3.3 a. (i) The student's t – test between study and Caucasian adults data.

The basis of this analysis was to compare the half-life mean of this study (children) data with that of healthy Caucasian adults to test for significant difference between the two groups or samples . The statistical null hypothesis is that the half life and hence the clearance of amodiaquine in Ghanaian children is equivalent to that of the healthy Caucasian adults. The details of this t-test are as shown below in table 3.4.a. (i).

Table 3.4.a. (i). Student's t-test between study and Caucasians data; ($t_{½}$) means.		
	STUDY DATA $t_{1/2}$	CAUCASIANS DATA $t_{1/2}$
Mean +/- SEM	4.8746 +/- 0.3691 hrs	5.200 +/- 1.7000 hrs
n	15	7
$SEM^2 = \sigma^2/n$	0.1362	2.8900

$\sigma d^2 = \sigma_1^2/n_1 + \sigma_2^2/n_2$	=0.1362 + 2.8900 = 3.0262
σd = SQRT (σd^2)	= SQRT (3.0262) = 1.7396
$t = (\bar{x}_1 - \bar{x}_2)/ \sigma d$	= (5.2 - 4.8746) / 1.7396 = 0.1871

Where; $\bar{x}_1$ is the mean half-life of the study sample (Ghanaian children),

$\bar{x}_2$ is the mean half-life of the literature sample (healthy Caucasian adults),

SEM is the standard error of the mean,

n is the number of replicates, observations, or sample size in each sample,

σ^2 is an estimate of variance; σd^2 is the variance of the difference between the two means,

$df = (n_1 + n_2 - 2)$ is the number of degrees of freedom.

From the standard ***t-tables***, the critical **t** value at probability level, p = 0.05 for 20 degrees of freedom, (i.e. $n_1 + n_2 - 2 = 20$) is 2.09. But the test statistic t or the calculated t value of 0.19 is less than this critical **t** (2.09). This implies that the study data is consistent with the statistical null hypothesis. The inference is that the half life and hence the clearance of oral amodiaquine in Ghanaian children with uncomplicated malaria is equivalent to that of healthy Caucasian adults. There is therefore, no significant difference between the two groups or sub populations as far as the pharmacokinetics of the drug is concerned.

3.3 a ii. Chi – squared test, (Yates correction) on study group and Caucasian data

The chi-squared test, (table 3.4 a. ii.) with modification by the Yates correction factor was conducted on the mean half life values of the study group and healthy Caucasian adults data (www.biology.ed.uk/statistics , {accessed 10 February 2009}), The statistical null hypothesis in this analysis is that the half life and hence the clearance of oral amodiaquine in Ghanaian children with uncomplicated malaria is equivalent to that of the healthy Caucasian adults. From the standard chi-squared tables, the critical $\boldsymbol{X^2}$ value

at probability level p = 0.05 and one degree of freedom, (df = 1), is 3.84. The test statistic X^2 or the calculated X^2 value of 2.85 in this analysis is lower than the critical $\boldsymbol{X^2}$ value. This implies that the study data is consistent with the statistical null hypothesis. Thus the difference in half life and hence the clearance of oral amodiaquine between the two sub populations is statistically not significant. It can therefore be reasonably inferred that there is no significant difference in the disposition of oral amodiaquine between the two groups or sub populations; (i.e. Ghanaian children with uncomplicated malaria and healthy Caucasian adults).

Table 3.4.a. (ii). Chi-squared analysis on study group and Caucasian adults data.

n	Observed O	Expected E	(O – E)	| O – E| - 0.5	{| O – E | - 0.5}^{2}	{| O – E | - 0.5}^{2} / E
1	6.7	5.2	1.5	1.0	1.00	0.192
2	5.6	5.2	0.4	-0.1	0.01	0.002
3	4.9	5.2	-0.3	-0.2	0.04	0.008
4	6.4	5.2	1.2	0.7	0.49	0.094
5	3.7	5.2	1.5	1.0	1.00	0.192
6	6.5	5.2	1.3	0.8	0.64	0.123
7	3.1	5.2	-2.1	1.6	2.56	0.492
8	4.5	5.2	-0.7	0.2	0.04	0.008
9	3.1	5.2	-2.1	1.6	2.56	0.492
10	3.0	5.2	-2.2	1.7	2.89	0.556
11	6.0	5.2	0.8	0.3	0.09	0.017
12	6.2	5.2	1.0	0.5	0.25	0.048
13	3.2	5.2	-2.0	1.5	2.25	0.433
14	6.3	5.2	1.1	0.6	0.36	0.069
15	3.9	5.2	-1.3	0.8	0.64	0.123
						SUM= X^2 = 2.851

The statistic or calculated X^2 value in this analysis was 2.85

3.3 a iii. G- test of goodness – of - fit on study and Caucasian adults data.

The pharmacokinetic parameter, half life and hence the clearance of oral amodiaquine in the study group was finally subjected to the G-test of goodness-of –fit relative to the

Caucasian adults data. The statistical null hypothesis in this analysis is that the half life and hence the clearance of oral amodiaquine in the study population is equivalent to that of the Caucasian adults. From the standard chi-squared tables, the critical $\boldsymbol{G}$ or $\boldsymbol{X^2}$ value analogous to the chi-squared analysis is 3.84 at probability level p = 0.05 and one degree of freedom, (df). The details of this analysis are shown in table 3.4 a. (iii) below.

Table 3.4.a. (iii). G- test on study and Caucasian adults data, ($t_{1/2}$).

n	Observed O	Expected E	(O/E)	Ln(O/E)	O*Ln(O/E)
1	6.7	5.2	1.29	0.25	1.68
2	5.6	5.2	1.08	0.08	0.45
3	4.9	5.2	0.94	-0.06	-0.29
4	6.4	5.2	1.23	0.21	1.34
5	3.7	5.2	0.71	-0.34	-1.26
6	6.5	5.2	1.25	0.22	1.43
7	3.1	5.2	0.59	-0.51	-1.58
8	4.5	5.2	0.87	-0.14	-0.63
9	3.1	5.2	0.59	-0.51	-1.58
10	3.0	5.2	0.58	-0.54	-1.62
11	6.0	5.2	1.15	0.14	0.84
12	6.2	5.2	1.19	0.17	1.05
13	3.2	5.2	0.62	-0.48	-1.54
14	6.3	5.2	1.21	0.19	1.19
15	3.9	5.2	0.75	-0.29	-1.13
					SUM = 1.64

The test statistic G or calculated X^2 in this analysis was 3.28. However, at probability level p = 0.05 and one degree of freedom, the observed critical **G** or $\boldsymbol{X^2}$ value of 3.84, is greater than the calculated G or statistic X^2. This observation implies that the study data is consistent with the statistical null hypothesis. The inference under these conditions is that there is no significant difference between the two sub populations as far as the half life and hence clearance i.e. pharmacokinetics of oral amodiaquine is concerned.

The statistical analyses above, namely the student's t-test, the chi-squared test and the

G-test of goodness of fit, seem to confirm the equivalence of the two groups or sub populations as far as the disposition or pharmacokinetics of oral amodiaquine is concerned. Thus there seem to be no significant difference between Ghanaian children with uncomplicated malaria and healthy Caucasian adults.

3.3. b (i). Student's t-test (analysis) between study group and Zambian adult's data

The student's t-test (analysis) was conducted on the half life means of this study data of Ghanaian children, and that of Zambia adults (Winstanley et al., 1990).

Table 3.4 b. (i). Student's t-test between study and Zambians data.

	STUDY DATA ($t_{1/2}$)	ZAMBIANS DATA $t_{½}$
Mean +/- SEM.	4.9 +/- 0.37	3.7 +/- 0.35
n	15	14
$SEM^2 = \sigma^2/n$	0.1369	0.1225
$\sigma d^2 = \sigma_1^2/n_1 + \sigma_2^2/n_2$	= 0.1369 + 0.1225 = 0.2594	
$\sigma d = SQRT (\sigma d^2)$	= SQRT(0.2594) = 0.5093	
$t = (x_1 – x_2) / \sigma d$	= (4.9 – 3.7) / 0.5093 = 2.35	

The main objective of this analysis was to test for significant difference of the half life means and hence elimination rate constant kel, of oral amodiaquine between the two sub populations. The statistical null hypothesis in this analysis is that, the half life and hence the elimination rate constant kel, of oral amodiaquine in Ghanaian children with uncomplicated malaria is equivalent to that of Zambian adults also with uncomplicated malaria. The details of this t-test analysis are as shown in table 3.4 b. (i) above. The statistic t or calculated t in this analysis was **2.35**. From the standard ***t-tables,*** the critical **t** value at probability level p=0.05 and degrees of freedom level; 27, (i.e. $n_1 + n_2 – 2 = 27$) is **2.05**. The statistic t or calculated t value of 2.35 is greater than the critical **t** value of 2.05. This observation implies that the study data is not consistent with the statistical

null hypothesis. It can therefore be reasonably inferred that the half life and hence the elimination rate constant of oral amodiaquine in Ghanaian children with uncomplicated malaria is not equivalent to that of the Zambian adults. Thus there is statistically, a significant difference in the disposition of oral amodiaquine between the two groups.

3.3 b. (ii) Chi – squared test on study group and Zambian adult's data

The chi – squared test for goodness of fit was conducted on the study group and the Zambian adult's data, [table 3.4 b (ii)].

Table 3.4. b (ii) Chi-squared test on study and Zambian adults data.

n	Observed (O)	Expected (E)	(O – E)	$(O – E)^2$.	$(O – E)^2 / E$
1	6.7	3.7	3.0	9.00	2.43
2	5.6	3.7	1.9	3.61	0.98
3	4.9	3.7	1.2	1.44	0.39
4	6.4	3.7	2.7	7.29	1.97
5	3.7	3.7	0.0	0.00	0.00
6	6.5	3.7	2.8	7.84	2.12
7	3.1	3.7	-0.6	0.36	0.10
8	4.5	3.7	0.8	0.64	0.17
9	3.1	3.7	-0.6	0.36	0.10
10	3.0	3.7	-0.7	0.49	0.13
11	6.0	3.7	2.3	5.29	1.43
12	6.2	3.7	2.5	6.25	1.69
13	3.2	3.7	-0.5	0.25	0.07
14	6.3	3.7	2.6	6.76	1.83
15	3.9	3.7	0.2	0.04	0.01
					SUM=X^2 = 13.41

The calculated X^2 or statistic X^2 value in this analysis was 13.41.

From the standard chi-squared tables, the critical $\boldsymbol{X^2}$ value at p = 0.05 for one degree of freedom (i.e. d.f = 1) is 3.84. This critical $\boldsymbol{X^2}$ value is far below the calculated X^2 or the statistic X^2 value. The statistical null hypothesis in this analysis is that the half life and hence the elimination rate constant kel of oral amodiaquine in Ghanaian children is equivalent to that of the Zambian adults. However, the test statistic X^2 or the calculated

X^2 value of 13.41 far exceeds that of the critical $\boldsymbol{X^2}$ value of 3.84 at p = 0.05 for one d.f. Furthermore, the statistic X^2 is even greater than the critical $\boldsymbol{X^2}$ value at probability level p = 0.001, (i.e. 10.83). This implies that the study group's data departs strongly from the statistical null hypothesis. It can therefore be reasonably inferred that the difference in half life and hence the elimination rate constant of oral amodiaquine, between the two sub populations (i.e. Ghanaian children with uncomplicated malaria and Zambian adults) is statistically, highly significant. The chi - squared test, analogous to the student's t-test, therefore confirms the significant difference in the disposition of oral amodiaquine between Ghanaian children with uncomplicated malaria and Zambian adults.

3.3. b. (iii). G – test of goodness – of – fit on study group and Zambian adult's data.

The pharmacokinetic data of the study was finally subjected to the G – test of goodness – of – fit analysis relative to the Zambian adult's data. The details of this test are shown in table 3.4 b (iii), page 80. The statistical null hypothesis in this analysis is that, the half life and hence the elimination rate constant kel, of the drug in Ghanaian children with uncomplicated malaria is equivalent to that of the Zambian adults. From the standard chi-squared tables, the critical G value or $\boldsymbol{X^2}$ value at probability level p = 0.05 for one degree of freedom, (d.f) is 3.84. However, from the ensuing analysis the G statistic or calculated X^2 value for the data is 46.14 which is far above the critical G or $\boldsymbol{X^2}$ value of 3.84. The calculated G or statistic X^2 value, even far exceeds the critical $\boldsymbol{X^2}$ value of 10.83 at probability level p =0.001 for one degree of freedom. This implies that the study group's data departs strongly from the statistical null hypothesis and that the difference between the two sub populations is highly significant. It can therefore be reasonably inferred that the difference in half life and hence the elimination rate constant of the drug

between Ghanaian children with uncomplicated malaria and that of the Zambian patients is highly significant. The G – test for goodness - of – fit therefore, like both the student's t – test and the chi – squared test, confirms significant difference in the disposition of oral amodiaquine between the two sub populations; thus Ghanaian children with uncomplicated malaria and Zambian adults.

Table 3.4 b. (iii). G-test on study and Zambian adults data.

n	Observed (O)	Expected (E)	(O/E)	Ln (O/E)	O*Ln(O/E)
1	6.7	3.7	1.81	0.59	3.953
2	5.6	3.7	1.51	0.41	2.296
3	4.9	3.7	1.32	0.28	1.372
4	6.4	3.7	1.73	0.55	3.52
5	3.7	3.7	1.00	0.00	0.00
6	6.5	3.7	1.76	0.56	3.64
7	3.1	3.7	0.84	-0.17	-0.527
8	4.5	3.7	1.22	0.19	0.855
9	3.1	3.7	0.84	-0.17	-0.527
10	3.0	3.7	0.81	-0.21	-0.63
11	6.0	3.7	1.62	0.48	2.88
12	6.2	3.7	1.68	0.51	3.224
13	3.2	3.7	0.86	-0.15	-0.48
14	6.3	3.7	1.70	0.53	3.339
15	3.9	3.7	1.05	0.04	0.156
					SUM = 23.07

The test statistic G or calculated X^2 value was [2 **.** (23.07)] = 46.14

All the statistical analyses, (namely the student's t-test, the chi-squared test and the G-test of goodness-of-fit), seem to support and thereby establish a significant difference in the disposition of oral amodiaquine between Ghanaian children with uncomplicated malaria and Zambian adults.

3.3. c. Statistical analyses on study males and females data.

The pharmacokinetic data obtained in both male and female patients employed in the

study were subjected to various statistical analyses. These included the student's t-test, the chi-squared test and the G-test for goodness-of-fit.

3.3. c. (i) Student's t-test between males and females mean half lives.

The student's t-test (analysis) was conducted between the mean half-life values obtained for the males and females patients employed in the study. The details of this analysis are shown in table 3.4. c.(i) below.

Table 3.4.c. (i). Student's t-test between males and females mean half lives.		
	Males data	Females data
Mean +/- SEM	6.28 +/- 0.11	3.71 +/- 0.27
n	7	8
SEM=STDEV/ sqrt (n)	0.11	0.27
$SEM^2 = \sigma^2/n$	0.01	0.07
$\sigma d^2 = \sigma_1^2/n_1 + \sigma_2^2/n_2$	0.01 + 0.07 = 0.08	
$\sigma d = sqrt (\sigma d^2)$	=sqrt(0.08) = 0.28	
$t = (x_1 - x_2) / \sigma d$	= (6.28 – 3.71) / 0.28 = 9.18	

The statistical null hypothesis in this analysis is that the mean half life (t ½) of oral amodiaquine in males is equivalent to that in the female patients. The above analysis indicated a calculated t or statistic t value of 9.18. From the standard ***t-tables***, a critical **t** value of 2.16 is obtained at probability level p = 0.05, for a number of degrees of freedom (d.f), value of 13, (i.e. n_1+n_2 -2). The statistic t value of 9.18 exceeds that of the critical value of 2.16 at this probability level of 0.05. Furthermore, at probability level of p = 0.001 a critical **t** value for 13 degrees of freedom of 4.22 is even lower than the statistic t value 9.18. The inference is that the data are not consistent with the null hypothesis. Therefore the difference in mean half life (t ½) values between the male and female patients employed in the study is statistically significant.

3.3. c. (ii). Chi-squared analysis on male and female data (half life values)

The statistical Chi-squared test was conducted on the half-life data of the males and females patients. The details of this analysis are shown in table 3.4.c. (ii) below.

Table 3.4 c. (ii). Chi-squared analysis on males and females t ½ data.					
n	Observed t ½ (O)	Expected t ½ (E)	(O – E)	$(O-E)^2$	$(O-E)^2$ / E
1	6.65	3.71	2.94	8.64	2.33
2	5.90	3.71	2.19	4.80	1.29
3	6.42	3.71	2.71	7.34	1.97
4	6.53	3.71	2.82	7.95	2.14
5	5.96	3.71	2.25	5.06	1.37
6	6.19	3.71	2.48	6.15	1.66
7	6.32	3.71	2.61	6.81	1.83
					SUM=X^2 = 12.60

The statistical null hypothesis in this analysis is that the mean half life value of the male patients is equivalent to that of the females. From table 3.4.c.(ii) above, the statistic X^2 or calculated X^2 value was 12.60. But from the standard chi-squared table, the critical $\boldsymbol{X^2}$ value at probability level p = 0.05 and one degree of freedom is 3.84. Comparison of these figures indicates that the statistic X^2 far exceeds the critical value. This is an indication that the data is not consistent with the statistical null hypothesis. It can therefore be reasonably inferred that statistically, there is a high significant difference between the mean half life values of the male and the female patients or subjects.

3.3. c. (iii). G-test on male's and female's data (half life values)

The G-test of goodness-of-fit analysis was conducted on the male's half life data relative to the mean half life value of the female patients. The details of this analysis are shown in table 3.4.c.(iii) below.

Table 3.4.c. (iii). G-test on males t ½ data and females mean t ½					
n	Observed (O)	Expected (E)	(O/E)	Ln(O/E)	O*Ln(O/E)
1	6.65	3.71	1.79	0.58	3.86

2	5.90	3.71	1.59	0.46	2.71
3	6.42	3.71	1.73	0.55	3.53
4	6.53	3.71	1.76	0.57	3.72
5	5.96	3.71	1.61	0.48	2.80
6	6.19	3.71	1.67	0.51	3.16
7	6.32	3.71	1.70	0.53	3.35
					SUM= 23.13

The Statistic G or calculated G was [2 **.** (23.13)] = 46.26.

The statistical null hypothesis is that the mean half life values obtained in both males and females patients are equivalent. From the standard chi-squared tables, a critical ***G*** or $\boldsymbol{X^2}$ value of 3.84 is obtained at probability level p = 0.05 for one degree of freedom. But a statistic G or calculated G value of 46.26 estimated in this analysis far exceeds the critical ***G***. Even a critical ***G*** value of 10.83 at probability level, p = 0.001 for one degree of freedom is observed to be further lower than the statistic G. The data therefore is not consistent with the statistical null hypothesis and that the difference between the mean half life values of the males and females data is highly significant.

The student's t-test, the chi-squared test and the G-test analyses, conducted on the males and females data seem to confirm a significant difference in the mean half life values of oral amodiaquine between the two groups.

From literature, the following pharmacokinetic parameters of amodiaquine in adults are documented (Krishna et al., 1990).

CL: 5.5 (1.6 – 17.3) L hr^{-1} kg^{-1}

V: 38.3 (3.7 – 127.9) L kg^{-1}.

kel: 0.13 (0.07 – 1.44) hr^{-1}.

Other authors have these pharmacokinetic parameter values on the average, with wide

interpatient PK variability to be;

CL; (2 – 20) L hr^{-1} kg^{-1}

V; (20 – 40) L $kg^{-1.}$

(www.impact-malaria.com , {accessed 24 January 2009}).

In this current study, if the volume of distribution V, is assumed to be constant among the patients, then the parameter clearance CL, in employed patients (i.e. Ghanaian children) may be estimated as follows;

$$CL = V.kel = (38.3) . (0.15) = 5.75 \text{ L } hr^{-1} \text{ } kg^{-1}.$$

The estimated mean elimination rate constant kel, value of 0.15 hr^{-1} in this study is similar to or within the range values published in literature (Krishna et al., 1990). This implies that a constant kel was observed in the studied patients of Ghanaian children. Based on the above relationship, an analogous constancy in Clearance parameter of oral amodiaquine in Ghanaian children is expected. Thus the Clearance value of 5.75 L hr^{-1} kg^{-1}, calculated above in Ghanaian children is within the adult literature range values of (1.6 – 17.3) L hr^{-1} kg^{-1}. This is an indication that all the Ghanaian children employed in the study generally exhibited a constant clearance of orally administered amodiaquine.

CHAPTER FOUR.

DISCUSSION, CONCLUSION AND RECOMMENDATIONS.

4.1. DISCUSSION AND CONCLUSION.

One of the main objectives of clinical pharmacokinetics is the study of drug disposition, or the processes of absorption, distribution, metabolism and elimination (ADME) of drugs in humans. It also includes the modification of these processes in various

physiopathological and clinical situations as well as dosing regimen adjustments or corrections and therapeutic implications (www.umanitoba.ca , {accessed 10 February 2009}). Therapeutic monitoring of the plasma or serum levels of drugs with narrow therapeutic margin permits, among other objectives, dosage individualization and hence optimization of therapy. Specific methods such as population pharmacokinetics and statistics, contribute powerfully to increasing precision in the estimation of individualized pharmacokinetics and hence dosage regimens (Bennette et al., 2005).

Generally, published literature on the pharmacokinetics of oral amodiaquine in the Sub-Saharan African region is limited or scanty. The drug has only been studied and defined in a few pharmacokinetic investigations in few subjects or patients. Virtually, no detailed pharmacokinetic studies of the drug involving different subjects in terms of age, gender, race, and varying methods of the drug analysis within the Sub-Saharan Africa region have been found in the literature. Available records indicate that only a few pharmacokinetic evaluation of oral amodiaquine in this region has been conducted. In the West African sub region for instance, pharmacokinetic investigations of the drug in only four Nigerian adults was found in the published literature (Winstanley et al., 1990). Therefore pharmacokinetic data of oral amodiaquine (AQ) in uncomplicated malaria patients as well as in healthy volunteers within this region is limited. The practice of deducing paediatric or children doses by adjusting adult doses for body surface area or body weight is often inadequate particularly for the antimalarials (King et al., 2002). A better understanding of the pharmacokinetic profile of oral amodiaquine in children would therefore facilitate or enhance its successful antimalarial therapy within this region. This current study is one of the premier reports of the pharmacokinetics of

amodiaquine (AQ) following oral administration in Ghanaian children with uncomplicated malaria of ages between 8 and12 years. The study employed only urine data analysis as there was no access to either blood or plasma samples.

There is limited literature documenting the absorption kinetics of oral amodiaquine (AQ) Most researchers examined the pharmacokinetics of desethylamodiaquine (DESQ), the principal metabolite of amodiaquine's rapid and extensive first pass effect following oral administration. In this current study, the method of residuals concept was applied to the excretion rate – time data or curve to investigate the absorption kinetics of the drug (Gabrielson and Weiner, 1994).The analysis resulted in the estimation of the following absorption pharmacokinetic parameters of the drug (i.e. absorption rate constant ka, and its corresponding absorption half-life t ½ a.). From table 3.3, the absorption rate constant ka, estimated at 95% CI ranged from 0.3586 to 0.5418 hr^{-1}. The mean absorption rate constant (ka) value estimated at 95% CI was 0.450 +/- 0.043 hr^{-1}; table 3.2. Estimate for the corresponding absorption half life (t ½ a) value at 95% CI ranged between 1.4129 and 2.0271 hrs; table 3.3. Moreover, from table 3.2, the mean absorption half-life (t ½ a) value at 95% CI was estimated as1.720 +/- 0.1435 hrs.

Generally, higher absorption rate constant, ka values of orally administered amodiaquine were observed in all the patients; (i.e. Ghanaian children with uncomplicated malaria.) Accordingly, the corresponding absorption half life, $t_{1/2}a$, values were also generally lower and thereby faster. The general high absorption rate constant (ka) values observed serves as a confirmation of literature assertion of amodiaquine's rapid absorption following its oral administration (Krishna et al., 1990). It can therefore be partly

concluded from the study that, following oral administration, the process of absorption of amodiaquine is rapid.

The data from the study indicates extremely low fe values (i.e. fraction of administered dose eliminated in the unmetabolized form in urine) of oral amodiaquine (AQ) in the patients. This is an indication of the drug's extensive first – pass metabolism by the hepatic system. This effect involves the biotransformation of amodiaquine (AQ) to various metabolites which includes the major metabolite desethylamodiaquine (DESQ). This principal metabolite, (DESQ) has been observed and thereby established to be more active, in vivo, than the parent drug (AQ) (White et al., 1990).

From table 3.3, individuals fe at 95% CI ranged between 0.0035 and 0.0083. A mean fe value of 0.0059 +/- 0.0011 at 95% CI was recorded; table 3.2. These observations of low fe values are further reflection of the extensive first-pass metabolism which amodiaquine (AQ) undergoes after oral administration. Extreme departure of fe value from the 95% CI range or limits may be attributed to some form of hepatic insufficiency on the part of the subject under investigation. In this current study no extreme departure of fe from the 95% CI range was observed. It may therefore be reasonably inferred that all the patients who participated in the study had no hepatic problems.

Generally, the parameter Clearance (CL), which is directly proportional to kel (i.e. CL = V**.**kel) and hence indirectly proportional to fe (i.e. fe = ke/kel), exerts a great influence on the overall elimination process or renal clearance of the kidneys. This concept or principle is therefore employed as a principal tool in the clinical renal function test for the kidneys. The clinical renal function test constitutes the basis for dosage regimen design or adjustment for renally impaired or insufficiency patients. It is hereby observed

that the pharmacokinetic parameter CL, and hence fe play a key role in this clinical procedure. The absolute value of fe may serve as a reflection of the bioavailability (F) of the drug under investigation. A higher fe value is an indication of high bioavailability and hence high levels of plasma concentrations of the drug under investigation.

In terms of secondary pharmacokinetic parameter values, detailed studies or published literature on oral amodiaquine in this region is virtually non-existing. However, in this current study, application of pharmacokinetic principles to the urinary excretion data led to the estimation of the parameters km and ke of the drug. Specifically, analysis of a fit of one-compartment model to the A.R.E. plot or curve, led to the estimation of the parameters fe and kel. From the pharmacokinetic relationship fe = ke /kel, the parameter ke was calculated. The parameter km was estimated from the relationship; kel = ke + km (www.boomer.org/c25 , {accessed 12 May 2007}). The km values, (i.e. the elimination rate constant of the fraction of administered dose eliminated in the metabolized form in urine) at 95% CI observed ranged from 0.1280 hr^{-1} to 0.1816 hr^{-1} with the mean 0.1548 +/- 0.012 hr^{-1}; tables 3.3 and 3.2 respectively. Ironically, the estimated mean km value of 0.1548 +/- 0.0125 hr^{-1} was almost identical to or equivalent to the estimated mean of the overall elimination rate constant kel value of 0.1553 +/- 0.0126 hr^{-1}. This is a further reflection of the drug's (i.e. oral amodiaquine) extensive metabolic clearance.

The secondary pharmacokinetic parameter ke was estimated. This is the elimination rate constant of the fraction of the administered dose eliminated in the unmetabolized form in urine. The ke values were calculated from the pharmacokinetic relationship;

kel = ke + km. At 95% CI limits the estimated ke values ranged between 0.0004 and 0.0012 hr^{-1}, table 3.3. From table 3.2 the estimated mean ke was 0.0008 +/- 0.0002 hr^{-1}. Generally, low ke values were observed in all the patients compared with the corresponding km values. These observations, once again indicate that amodiaquine undergoes extensive metabolic clearance and that its renal clearance is relatively low. On the basis of the above discussions, it may be included as part of the general conclusion from the current study that, following oral administration amodiaquine undergoes extensive hepatic first-pass effect.

Other pharmacokinetic (PK) parameters of oral amodiaquine of valuable clinical importance estimated were the elimination rate constant kel, and its corresponding elimination half life (t ½). The urinary excretion rate data obtained was subjected to non-compartmental model analysis from which the elimination rate constant kel, of oral amodiaquine (AQ) was calculated. From table 3.3, the estimated elimination rate constant kel, value at 95% CI range was between 0.1283 and 0.1823 hr^{-1}. The mean value for this parameter at 95% CI was estimated as 0.1553 +/- 0.0126 hr^{-1}; table 3.2. The corresponding elimination half-life (t ½) value of the drug was calculated by employing the pharmacokinetic relationship: t ½ = 0.693 / kel. From table 3.3, estimates for the elimination half- life (t ½) value of amodiaquine at 95% CI ranged from 4.0845 to 5.6645 hrs. The mean elimination half-life (t ½) value of the drug at 95% CI was estimated as 4.8746 +/- 0.3691 hrs; table 3.2.

The pharmacokinetic parameters kel, and t ½ values obtained were statistically compared, in separate analysis, with those published in literature in healthy Caucasian adults and

Zambian adult's with uncomplicated malaria. This was accomplished by employing the data from Winstanley et al (1987) , on both healthy Caucasian adults and Zambian adults with uncomplicated malaria as comparators for the results from this current study. Statistical analysis conducted between the study and healthy Caucasian adult's data indicated no significant differences in the disposition or pharmacokinetics of the drug in the two sub populations. All the statistical tests, namely the student's t-test, table 3.4a.(i). the chi-squared test, table 3.4 a (ii), and the G- test table 3.4 a (iii) carried out on these data indicated no significant difference between them. Thus the pharmacokinetic parameters of oral amodiaquine estimated in the study were statistically, similar to those published in literature for healthy Caucasian adults. It can therefore be reasonably inferred that there is no significant difference between Ghanaian children with uncomplicated malaria and healthy Caucasian adults. The observations further imply that age factor does not seem to affect or exert any considerable influence on the disposition of or the pharmacokinetics of orally administered amodiaquine. The occasionally observed adverse reactions or effects of orally administered amodiaquine in the country are usually experienced throughout the entire population. Thus subjects or patients of all ages do occasionally experience these adverse effects after oral administration of the drug. This is a further support of the independent nature of the pharmacokinetics or disposition of oral amodiaquine on age. Thereby it may be partly concluded that the pharmacokinetic parameter values of oral amodiaquine estimated in Ghanaian children with uncomplicated malaria were similar to those published in literature in healthy Caucasian adults.

However, in contrast to the above observations, statistical comparison of this present

study data with that of Zambian adult's with uncomplicated malaria led to revelation of significant differences in the pharmacokinetics of orally administered amodiaquine between the two sub populations or groups.

From available literature (Winstanley et al., 1990), the half life (t ½) values of oral amodiaquine in fourteen (14) Zambian adults were, mean 3.7 hrs, range (2.9 – 4.5) hrs at 95% CI limits. The corresponding elimination rate constant (kel), values at 95% CI were, mean 0.19 hr^{-1}, range (0.15 – 0.24) hr^{-1}. In this current study of Ghanaian children the half life (t ½) values were, mean 4.9 hrs, range (4.0 – 5.8) hrs; and the corresponding elimination rate constant kel values were, mean 0.15 hr^{-1}, range (0.12 – 0.17) $hr^{-1.}$ These were estimated at the 95% CI limits.

The observed significant differences between these sets of data were confirmed and thereby established by all the statistical analyses carried out on them. These were the student's t-test, table 3.4 b. (i), the chi - squared test, table 3.4.b. (ii) and the G- test for goodness-of-fit, table 3.4.b (iii). Essentially, the estimated mean elimination rate constant, kel value of 0.15 hr^{-1} in this current study data was statistically observed to be significantly lower than that of 0.19 hr^{-1} in Zambian adults with uncomplicated malaria. Subsequently, by employing the pharmacokinetic relationship, t ½ = 0.693/kel, the corresponding elimination half-life (t ½) values in Ghanaian children as well as that in Zambian patients were estimated. The estimated mean elimination half-life (t ½) value of oral amodiaquine in Ghanaian children of 4.9 hrs was statistically observed to be significantly higher than that of 3.7 hrs in the Zambian adults. This implies that the possibility of a manifestation of the drug's potential adverse effects or reactions within the study population of Ghanaian children would be relatively higher than that in the

Zambian sub population. Therefore the occasional observation of adverse effects or reactions following oral administration of the drug in the country may in part be attributed to this significantly higher or longer mean half life (t ½) value of amodiaquine within the Ghanaian sub population. These observations are suspected to be principally due to the genetic or hereditary differences between the two groups. Other possible factors may include dietary, environmental, geographical and demographical differences.

With gender considerations within the current study group, a general pattern in elimination rate constant kel and hence half life (t ½) values among both parties were observed. Statistical analyses carried out on the mean half life (t ½) values of these data, indicated significant differences between the male and the female patients. Thus the statistical analyses employed namely, the student's t-test, table 3.4.c. (i), the chi-squared test, table 3.4.c. (ii) and the G-test, table 3.4.c. (iii), indicated and thereby confirmed a highly significant difference between the mean half life (t ½) values of the male and female Ghanaian children. The general observations made, among others include the following. Elimination rate constant kel values were observed to be statistically higher in the females than in the males. The estimated mean elimination rate constant kel value of 0.19 hr^{-1}, in the female group was significantly higher than the mean kel value of 0.11 hr^{-1} in the males. Subsequently, the corresponding elimination half-life (t ½) values in female patients were statistically lower than those in the males, table 3.2. Specifically, the mean elimination half life (t ½) value of 6.28 hrs estimated in male patients was statistically higher than the mean half life (t ½) value of 3.71hrs observed in the female data. Thus a significant difference between the mean half life (t ½) values of oral amodiaquine in male and female data was established. These differences which were

statistically highly significant may principally be due to differences in physiological composition, probably variations in sexual hormonal characters and levels.

One major limitation of the study was the failure to estimate the primary pharmacokinetic parameters clearance CL, and volume of distribution V. This was due to the inability to gain access to plasma samples. In addition to the elimination rate constant kel, and half life (t ½) estimated, other pharmacokinetic parameters which could be estimated from plasma data include the area under the plasma concentration - time curve AUC, C_{max}, and t_{max}. Therefore, for a better understanding of the disposition of oral amodiaquine and hence an optimization of its use in the country, there is the need for a further and extensive pharmacokinetic investigations involving plasma data in Ghanaians.

The observed and established significant difference in half-life (t ½) values of oral amodiaquine between Ghanaian children and Zambian adults may be a contributory factor to the adverse reactions which are occasionally experienced following its administration in the country. Specifically, the mean half-life (t ½) value of 4.9 hrs estimated in Ghanaian children was significantly higher than the value of 3.7 hrs in the Zambian adults. This probably implies that the average plasma concentration of the drug at the steady state is significantly higher in the Ghanaian data than in the Zambians. Therefore, a reduction in the plasma concentration of the drug in the Ghanaian population may effectively reduce some of these occasionally observed adverse effects. The reduction in plasma concentrations of the drug could be pharmacokinetically effected either by reducing the dose or by increasing the dosing interval, tau, of administration. Furthermore, it could be effected by applying both processes

simultaneously.

The pharmacokinetic parameter, half-life (t ½) and hence elimination rate constant, kel play significant roles in the adjustment of dosage regimens of drugs in patients under different physiopathological and or clinical conditions. These parameters thereby influence the dosing regimen of the drug under investigation within the population. The observed significant difference of these PK parameters between Ghanaian children and Zambian adults data may therefore further imply the need for separate dosing regimen of the drug among the two sub populations. There is the need for further and extensive pharmacokinetic investigations to substantiate this assertion. Subsequently, the currently available World Health Organization's (W.H.O.) recommended dosing regimen of oral amodiaquine in the country, which is based on pharmacokinetic studies in East African subjects might be inappropriate or misleading. Therefore to optimize the therapeutic use of the drug in the country, it appears there is the need for re-evaluation and adjustment of its currently available dosing regimen.

It may therefore be finally concluded that, for a more effective dosing or optimization of oral amodiaquine therapy in the country, there is the need for a downward adjustment of its dosing regimen. It is anticipated that such an adjustment based on pharmacokinetic principles, could minimize or reduce some of the adverse reactions which are occasionally experienced following oral administration of the drug in the country.

4.2 RECOMMENDATIONS.

From the results of this study, the following recommendations may be suggested. That;

a. Optimization of amodiaquine therapy in the country requires further and extensive pharmacokinetic studies or investigations of the drug in Ghanaians .

b. Further pharmacokinetic investigations or studies of oral amodiaquine in the country should be based on both urine and plasma data. It must include estimation and evaluation of other valuable parameters such as; clearance CL, volume of distribution V, area under the curve AUC, Cmax, and tmax. Moreover, pharmacokinetic studies involving larger sample sizes and sites throughout the entire country are recommended .

c. Pharmacokinetic analytical results and or information that would be obtained from investigations or studies involving plasma data in Ghanaians may be used in the adjustment of the currently available dosing regimen of oral amodiaquine in the country. This would ensure optimization of the therapeutic use of the drug in the country.

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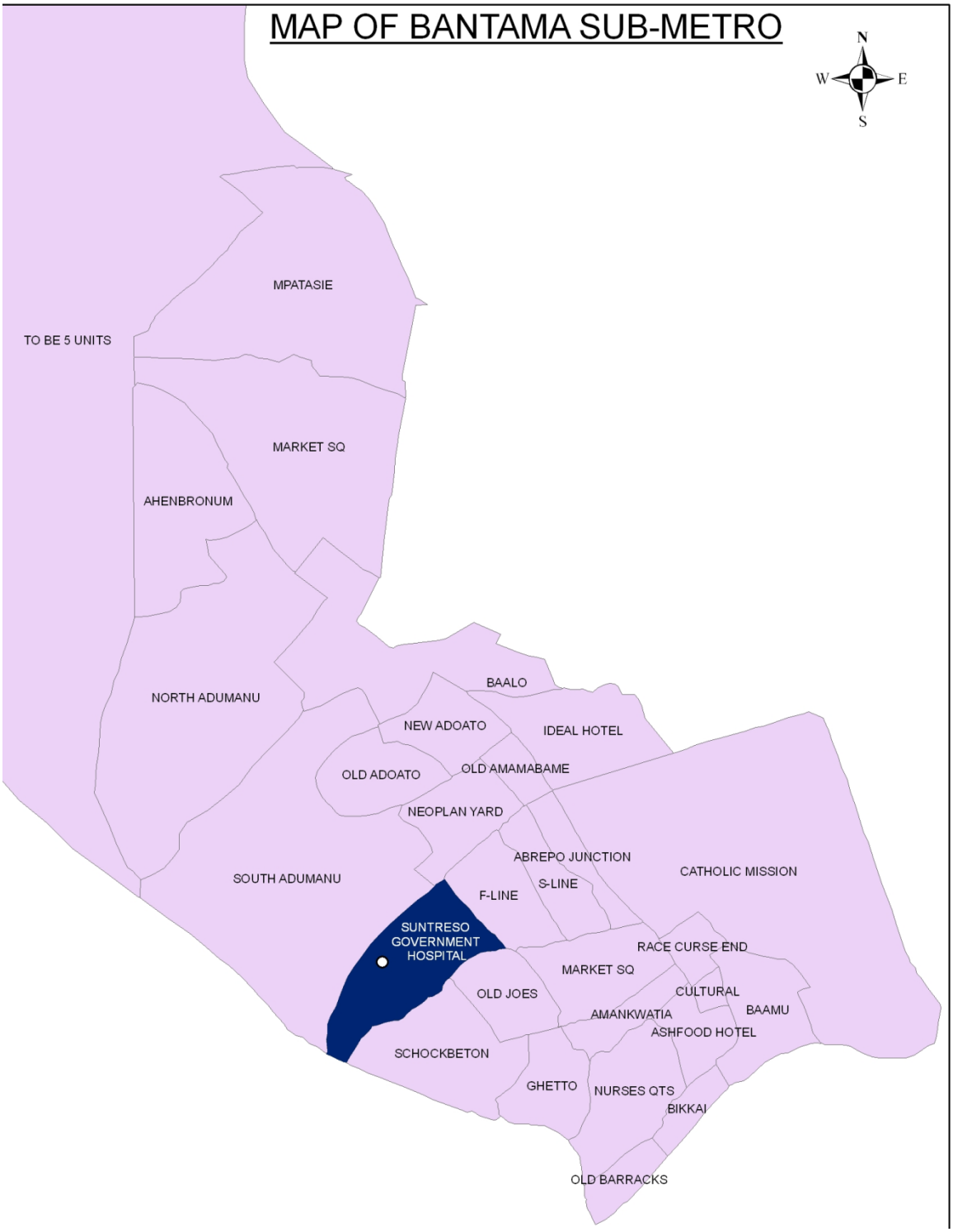
APPENDIX 1: SITE MAP OF SUNTRESO GOVERNMENT HOSPITAL.



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