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D-optimal design of a pediatric pharmacokinetic study of a fixed dose combination product of rifampicin-pyrazinamide-isoniazid for the treatment of tuberculosis.

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Ευχαριστίες

Θα ήθελα να ευχαριστήσω τον επιβλέποντα καθηγητή μου, κ. Αριστείδη Δοκουμετζίδη, για την πολύτιμη βοήθειά του καθ' όλη την διάρκεια της εκπόνησης της διπλωματικής μου εργασίας, καθώς και την κ. Σύψα και τον κ. Πανταζή που δέχτηκαν να είναι στην τριμελή επιτροπή. Επίσης, θα ήθελα να ευχαριστήσω την οικογένεια μου και ιδιαιτέρως την γιαγιά μου, τον Γιάννη και τον φίλο μου τον Νίκο για την στήριξη και κατανόηση τους.

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CHAPTER 1

1.TUBERCULOSIS

1.1 DISEASE AND SYMPTOMS

Tuberculosis (TB) is one of the most well-known and contagious diseases worldwide. It is caused by the bacterium Mycobacterium tuberculosis, which first discovered by the German microbiologist Robert Koch in 1882. The main organ of the body that is primarily affected by the disease is the lungs. For this reason, this kind of the disease is called pulmonary tuberculosis. It can also be spread to other organs of the body, such as kidneys, brain and spine known as extra-pulmonary tuberculosis. It is spread from person to person through the air, so the most common way to insert the body is through inhalation of air droplets from a cough, sneeze or spit of an infected person.
According to the Centers for Disease Control and Prevention (CDC), there is a distinction between two kinds of tuberculosis infection: latent and active. Latent tuberculosis called the situation where the bacteria remain in the body in an inactive state. At this state there are no symptoms and the bacteria are not contagious, but they can become active. In active tuberculosis the bacteria do cause symptoms and can be transmitted to others. Common symptoms of active lung tuberculosis are cough with sputum and blood at times, chest pains, weakness, weight loss, fever and night sweats.

1.2 HISTORY FACTS

Tuberculosis has plagued humans since antiquity. During the 19^{th} and early 20^{th} centuries, the so-called pre-antibiotic era, tuberculosis was considered one of the deadliest diseases worldwide. Also known as “consumption,” “phthisis,” or the “white plague” was the cause of more deaths in industrialized countries than any other disease during this period. Between 70% and 90% of the urban populations of Europe and North America were infected with the TB bacillus, and about 80% of those individuals who developed active tuberculosis died of it. Around the 1880s, in an attempt to cure tuberculosis naturally and prevent its spread, infected people moved to sanatoria, quiet and isolated environments, where the air was pure and freely circulating. Other methods which were used to cure the fatal disease were gold,

arsenic, cod liver oil, herbs, bed rest, sunshine, etc. but none of them were really effective. (Birnbaum et al. 1891)

With the discovery of streptomycin in 1943, the first antibiotic which used for the treatment of tuberculosis, series of possible anti-tuberculosis drugs (isoniazid, ethambutol, rifampin, etc.) were introduced to clinical practice during the period 1940s-1970s. (Hedy 1972, Murray et al. 2015).The implementation of these drugs to TB treatment immediately resulted in a sharp decline of TB incidence throughout the world. At this period, it was commonly thought that TB was no longer a public health concern in many countries. Nonetheless, the disease came back in the 1980s. Not only the onset of multi-drug resistant tuberculosis (MDR-TB) play a vital role for that, but also the spreading epidemic of the acquired immune deficiency syndrome (AIDS) is associated with the outbreak of tuberculosis.(Glynn 1998)

Nowadays, tuberculosis is a preventable and curable disease. Although the disease's rates are decreasing in the United States, still remains one of the top ten causes of death worldwide. According to World Health Organization (WHO) key facts, tuberculosis is a leading killer of HIV positive people in developing countries, such as West and sub-Saharan Africa, Afghanistan, Pakistan, and India. It is estimated that in 2016, 10.4 million people fell ill with tuberculosis and 1.7 million died from the disease. Children (0-14 years of age) represent about 10-11% of all tuberculosis cases. In 2016, 250.000 children died of tuberculosis including children with HIV associated tuberculosis. Nevertheless, between the time period 2000 and 2016, due to anti-tuberculosis treatment system, around 53 million people were saved through diagnosis and treatment.

1.3 TREATMENT

1.3.1 NEED OF FIXED DOSE COMBINATION DRUGS

By the term mono-therapy, we are referring to the treatment of a particular disorder or disease with a single drug. Contrary to mono-therapy, in combination therapy, more than one medication is received by the patient in order to treat a single disease. A combination drug, which include two or more active pharmaceutical ingredients (APIs) combined in a single dosage form, is known as fixed-dose combination drug (FDC).

The transition from single-drug formulations to FDC tablets for the treatment of tuberculosis has been in process for many years, as the idea of using FDC tablets arose from the fact that tuberculosis always requires multidrug therapy. Since the late 1980's, two and three-drug FDC tablets have been used worldwide and are registered in more than 40 countries. Indeed, approximately one fourth of the TB cases world-wide receive treatment with rifampicin-containing FDC tablets. (WHO 1999) However, the large number of different strengths of the available FDCs creates confusion and the potential for incorrect dosing. FDCs were a matter of concern in the treatment of tuberculosis as well, as substandard FDCs and relatively poor bioavailability of rifampicin were documented in the global market. (Pillai et al. 1999, Laserson et al. 2001) A 1998 WHO survey of the global market for FDCs showed that there is a significant number of such combinations available in the market, but with very little consistency in dose formulation. In fact, most of these preparations do not conform to the WHO dose specifications. In 1999s WHO and the International Union against Tuberculosis and Lung Disease (IUATLD) as an additional step to ensuring proper treatment, recommended to replace single-drug formulations for the treatment of tuberculosis and the standardization of the appropriate doses and strengths of FDC tablets.

The justification for recommending that FDC tablets replace single-drug tablets as the primary treatment for tuberculosis includes the following factors:

- FDCs prevent mono-therapy, and it is expected that this will reduce the emergence of drug resistant tuberculosis. If mono-therapy is prevented, the risk for selection of drug resistant bacilli is reduced. If given unsupervised,

FDC tablets do not prevent patients from interrupting treatment repeatedly Multiple interruptions of treatment can lead to emergence of drug resistance

- FDCs simplify treatment, and thus minimize prescription error as fewer tablets required, and increase patient and doctor compliance
- FDCs simplify drug stock management, shipping and distribution
- FDCs reduce the risk of misuse of rifampicin for conditions other than tuberculosis. (Blomberg 2001)

1.3.2 TREATMENT FOR ADULTS

As regards the appropriate treatment for adults, the updated *Guidelines for treatment of drug-susceptible tuberculosis and patient care* provide recommendations based on newly emerged evidence on the treatment of drug-susceptible TB and patient care. Tuberculosis can be cured within a time period from 6 to 9 months. Of the approved drugs, the most common first-line anti-TB agents are

- Isoniazid (INH or H)
- Rifampicin (RIF or R)
- Ethambutol (EMB or E) and
- Pyrazinamide (PZA or Z).

Although the above mentioned antimicrobial drugs used against the tuberculosis disease since years ago as we previously mentioned, the disease cannot be treated effectively due to incorrect use of antimicrobial drugs or use of ineffective formulations of drugs (such as use of single drugs, poor quality medicines or bad storage conditions).Moreover, the bacteria that cause tuberculosis can develop resistance to these antimicrobial agents. Multi-drug resistant tuberculosis (MDR-TB) means that the disease does not respond to at least isoniazid and rifampicin, the two most powerful anti-tuberculosis drugs. Due to these reasons, it was an emergency to find a new fixed combination product so as to improve the up to now treatment.

Anti-tuberculosis treatment is divided into two phases: an intensive (initial) phase and a continuation phase. The purpose of the intensive phase is to rapidly eliminate the

majority of organisms and to prevent the emergence of drug resistance. The intensive phase uses more drugs. The purpose of the continuation phase is to eradicate the dormant organisms. Fewer drugs are generally used in the continuation phase because the risk of acquiring drug resistance is low, as most of the organisms have already been eliminated. For adults, a 6-month regimen of 2HRZE/4HR is recommended in order to treat new pulmonary tuberculosis patients with drug-susceptible TB. This regimen includes specifically2 months administration of isoniazid, rifampicin, pyrazinamide and ethambutol, followed by 4 months phase of continuation of treatment with isoniazid and rifampicin. If the 8-month regimen of 2HRZE/6HE (i.e. 2 months of isoniazid, rifampicin, pyrazinamide and ethambutol, followed by 6 months of isoniazid and ethambutol) for such patients is still in use, it is recommended that it be phased out.

1.3.3 TREATMENT FOR CHILDREN

Tuberculosis is an important health problem for pediatric population, too. However, there was no appropriate first-line tuberculosis treatment designed for children until recently. Inappropriate doses by cut or crushed pills were given to children as pediatric dosing regimens. These regimens were usually empirically derived from adult data, using linear extrapolation based on body weight and the guidelines relied on clinical experience instead of controlled trials. The issue was that children differ from adults in their response to drugs. WHO recognized that children population was administered with insufficient doses, especially for isoniazid and rifampicin. As a consequence of under-dosing medication, drug resistance and therapeutic failure occurred.(Donald PR et al.2011, Schaaf et al. 2005)

The main reasons for non-existence of appropriate dose regimen for anti-tuberculosis treatment in children are both ethical and practical. First of all, to date, pharmaceutical companies hesitated to spend money in pediatric drug research and development of fixed dose combination drugs for children due to limited market. A second reason was that before the production of a new FDC anti-tuberculosis drug, clinical studies have to be performed in pediatric population. Both for industry and for academic researchers, performing studies in children is very challenging due to ethical and practical reasons. Unlike clinical trials in healthy adults, research in healthy children is considered to be unethical. For this reason, all pediatric studies are performed in

patients. In all clinical trials, informed consent has to be signed by the patient before he or she can be enrolled into a trial. In pediatric trials, fully informed consent should be obtained from the parents or the legal guardian of the pediatric subject. Except for ethical challenges, practical matters also occur when conducting studies in children. (De Cock et al. 2011). According to the guideline *Clinical Investigation of Medicinal Products in the Pediatric Population Guidance* of European Medicines Agency, special measures are needed not only to protect the rights of pediatric study participants but also to shield from undue risk. The number of sampling times and the volume of blood withdrawn should be minimized in pediatric population. For this reason, several techniques are encouraged from Medical Agencies (European Medical Agency and FDA).One of these techniques is the use of population pharmacokinetic studies and sparse sampling design based on sampling theory so as to minimize the number of samples obtained from each patient. Generally, in sparse sampling approaches a patient should be contribute as few as 2 to 4 observations at the predetermined times to an overall population AUC. Another complicating factor is the limited available number of subjects that suffer from the same disease.

After sustained advocacy and new investment, the dose regimen recommendation in children was amended in 2010 and the dose of all the first-line anti-TB drugs increased. The recommended doses for treatment of children with TB differ compared to treatment of adults. Current guidance of the WHO for the treatment of children with tuberculosis is based on the last scientific evidence and recommends the use of fixed-dose combinations drugs. (Global tuberculosis report 2017) In TABLE 1. , the recommended first-line drug dosages of anti-TB treatment for children are presenting and should be used daily in children.

TABLE 1. RECOMMENDED FIRST-LINE DRUG DOSAGES FOR CHILDREN AS CURRENTLY RECOMMENDED BY WHO

Drug	Currently recommended daily dose (dose range) mg/day	Maximum Dose mg/day
Isoniazid	10 (7–15)	300
Rifampicin	15 (10–20)	600
Pyrazinamide	35 (30–40)	-
Ethambutol	20 (15–25)	-

* As children approach a body weight of 25 kg, adult dosages can be use

Children with suspected or confirmed pulmonary TB or TB peripheral lymphadenitis who live in settings with low HIV prevalence or low prevalence of isoniazid resistance and children who are HIV-negative, can be treated with a three-drug regimen consists of a two-month intensive phase with isoniazid, rifampicin, pyrazinamide (HRZ) followed by a two drug (HR) regimen for 4 months at the dosages specified in TABLE 1.

For many years, ethambutol was not recommended, but contraindicated, for use in young children (<5 years of age). The concern was that ethambutol might cause optic neuritis in children who were too young to report the early visual symptoms, which could thus lead to irreversible blindness. (Graham 2015) Nowadays, ethambutol should be added in the intensive phase for children with extensive disease or living in settings where the prevalence of HIV or of isoniazid resistance is high.

These days, formulations that do not need to be cut or crushed to achieve an appropriate dose are available, offering the opportunity to simplify and improve treatment for children. The formulations were developed in line with the revised 2014 WHO Guidance for national tuberculosis programs on the management of tuberculosis in children.However, even these formulations are not properly qualified medicinal products according to EMA or FDA standards and have not been tested clinically, therefore cannot be used in Europe or in the US. These formulations have been approved only through the WHO prequalification programme and are intended to be used in deprived countries. So, in fact Europe and the US are still uncovered in

terms of the availability of first-line paediatric fixed dose combination products for TB.

The child-friendly formulations (FIGURE 1.) currently available

- for *the intensive phase* of TB treatment are rifampicin 75 mg, isoniazid 50 mg and pyrazinamide 150mg and
- for *the continuation phase* of TB treatment are rifampicin 75mg and isoniazid 50mg

FIGURE 1. FIXED DOSE COMBINATION DISPERSIBLE TABLETS FOR THE TREATMENT OF TB IN CHILDREN.

The following dosing table provides information on the number of daily tablets needed to reach the proper dosing, based on the child's weight.

TABLE 2.NUMBER OF DAILY TABLETS BASED ON THE CHILD'S WEIGHT.

Weight Bands (kg)	Number of tablets Intensive phase RHZ 75:50:150	Intensive phase E 100	Number of tablets Continuation phase RH 75:50
4-7	1	1	1
8-11	2	2	2
12-15	3	3	3
16-24	4	4	4
≥25	Adult dosages recommended		

R = rifampicin; H = isoniazid; Z = pyrazinamide; E = ethambutol

1.4 SCOPE OF THE THESIS AND OUTLINE

As we previously mentioned, the design and conduct of a pediatric clinical trial may be costly, unethical and impractical to be implemented. For these reasons, even though in drug development the use of modeling and simulations is still limited, it could improve the design of clinical trials and reduce the cost. The purpose of this thesis is to design a pediatric pharmacokinetic clinical trial with a common sparse sampling design for rifampicin, isoniazid and pyrazinamide simultaneously using D-optimal design. This clinical trial if implemented, can be used to obtain Market Authorization for new first-line paediatric fixed dose combination products for TB in Europe which are currently lacking.

The remaining of this thesis proceeds as follows:

- Chapter 2: A brief introduction to pharmacometric theory, a detail description of the main pharmacokinetic parameters and models is given. Finally, an introduction to the population pharmacokinetic modeling and the non-linear mixed effects models theory is given.
- Chapter 3: a basic mathematical theory based on Fisher Information Matrix and D-optimal Design and a description of model evaluation techniques.

- Chapter 4: a presentation of our work; the design of a sparse pediatric pharmacokinetic study with D-optimal design for the treatment of tuberculosis.

CHAPTER 2

2. PHARMACOMETRICS

According to Food and Drug Administration, pharmacometrics is an emerging science defined as the science that quantifies drug, disease and trial information to aid efficient drug development and regulatory decisions. Pharmacometrics uses mathematical models based on biology, pharmacology, physiology and disease for quantitative analysis of interactions between drugs and patients. Several kind of studies and respective models, belong to the field of pharmacometrics. These are:

- Pharmacokinetic (PK) studies which with the use of pharmacokinetic models describe the drug concentration-time courses in body fluids resulting from administration of a certain drug dose. In other words, pharmacokinetic studies describe what the body does to a drug.
- Pharmacodynamic (PD) studies which with the use of pharmacodynamic models describe the observed effect (response) resulting from a certain drug concentration when it enters the body or, what a drug does to the body.
- Physiologically based pharmacokinetic (PBPK) models consist of a series of mathematical representations of biological tissues and physiological processes in the body and are designed to predict an internal dose at target organs for risk assessment applications. **(**Peters S.A. ,2012)
- Exposure-response models describe the relationship between exposure (or pharmacokinetics), response (or pharmacodynamics) for both desired and undesired effects
- Disease models describe the relationship between biomarkers and clinical outcomes, time course of disease and placebo effects. (Mould and Upton, 2012)

As in this thesis only PK models are going to be used, a further explanation of pharmacokinetic process is given in detail below.

2.1 PHARMACOKINETICS

2.1.1 ADME PROCESS

Pharmacokinetic studies assess the fate of the drug from the time it enters the living organism and their effects in the body. Pharmacokinetic process consists of four phases; the absorption, the distribution, the metabolism and the excretion phase. This process often referred to as ADME process. An illustration of ADME process is seen in FIGURE 2.This process determines when the drug appears in the blood stream and for how long it remains there. In order for a drug to cause a therapeutic response, it must reach adequate concentrations in the blood so that it can reach and interact with drug receptors in adequate numbers to trigger a noticeable action. The course of drug action is, therefore, directly correlated with the concentration of the drug in the blood stream, and is dependent upon the ADME processes.

More specifically:

- The absorption is the movement of a drug from its site of administration (e.g. oral administration, intravenous administration, sublingual administration e.tc.) to the bloodstream. The rate and extent of absorption depends on the route of administration, the formulation and chemical properties of the drug, and physiologic factors that can impact the site of absorption.
- The distribution is the transportation of a drug often via the bloodstream, to its site of action. From there, the drug may distribute into muscle and organs, usually to differing extents. Blood flow to different organs of the body is not equal. The most vitally important organs of the body receive the greatest supply of blood such as the brain, the liver and the kidneys.
- The metabolism refers to a process whereby the body converts a drug that has been absorbed by the body from its original form and into a new form, called metabolite. The most important site of drug metabolism is the liver.
- The excretion refers to the removal of drug from the body usually through the kidneys (urine).The complete removal of the drug from the body is referred to as elimination. Elimination of the drug encompasses both the metabolism of the drug, and excretion of the drug through the kidneys.(Sakai, 2008)

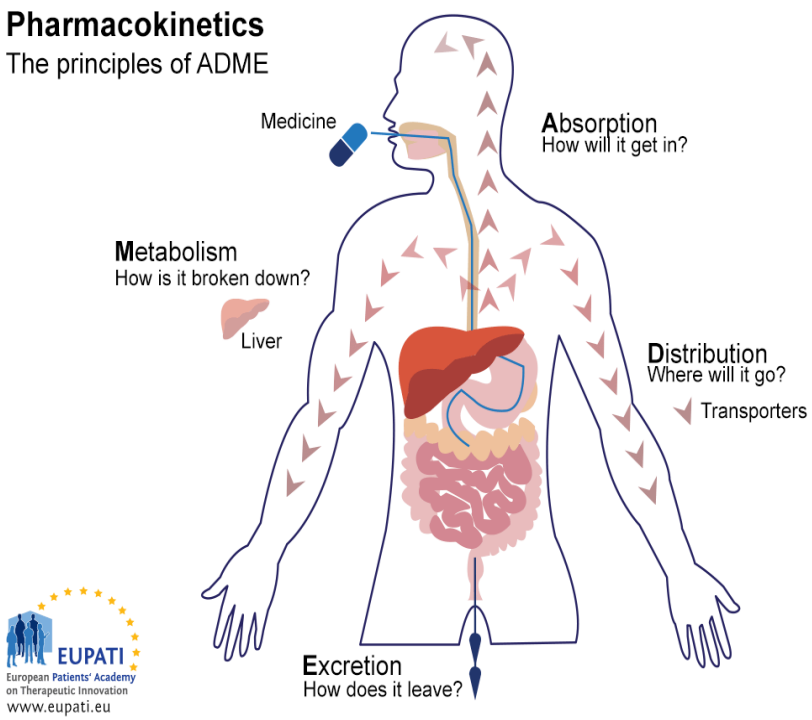


FIGURE 2. ADME PROCESS

2.1.2 PHARMACOKINETIC PARAMETERS

In this section, a description of the main pharmacokinetic parameters is given. (AGAH working group pharmacokinetics, 2004)

Dose (D)

Dose is defined as the amount (A) of drug administered in the body. It is measured in amount units. A common dose measurement is *mg*.

Volume of distribution (V)

Volume of distribution (V) is often referred as the apparent volume of distribution. It is defined as the volume of plasma in which the total amount of drug in the body would be required to be dissolved in order to reflect the drug concentration attained in plasma. It is measured in volume units (e.g.L).

Concentration (C)

Concentration is the amount of drug in a given volume of plasma, described by the formula $C = \frac{A}{V}$ and is measured in amount/ volume units. (e.g.mg/L)

Cmax

Cmax is defined as the observed maximum plasma or serum concentration after drug administration.

tmax

tmax is defined as the time which the drug needs to reach Cmax and is measured in time units (e.g.h).

tlag

tlag ,or lag-time , is the time delay between drug administration and first observed concentration above LOQ (Limit of Quantification) in plasma.

Area under the curve (AUC)

Area under the curve is the area under the concentration-time curve from zero up to ∞ with extrapolation of the terminal phase. The area under the curve is the definite integral in a plot of drug concentration in blood plasma vs. time. In practice, the drug concentration is measured at certain discrete points in time and the trapezoidal rule is used to estimate AUC. The AUC represents the total drug exposure over time.

Bioavailability

Bioavailability refers to the fraction of dose which enters systemic circulation, thereby accessing the site of action. Bioavailability can be measured in terms of "absolute bioavailability" or "relative bioavailability ".

Absolute Bioavailability(F)

Absolute bioavailability (in %) is the amount of drug from a formulation that reaches the systemic circulation relative to an intravenous (IV) dose. The IV dose is assumed to be 100% bioavailable, or F=100%.

Relative Bioavailability(F_{rel})

Relative bioavailability (in %) is the amount of drug from a formulation that reaches the systemic circulation relative to a different formulation (non-IV) such as oral solution. Relative bioavailability compares the bioavailability between two different dosage forms and calculated by F_{rel}= f_{rel} x 100, where f_{rel} is the fraction of the administered dose in comparison to a standard (non- IV) and calculated by $f_{rel} = \frac{AUC\ x\ Dstd}{AUCstd\ x\ D}$, std=standard.

Rates

In order to describe the processes of ADME, the rates of these processes are described below. By the term of rate, we define the velocity at which each process proceeds.
The rates of ADME include the absorption rates and elimination rates.

Absorption Rate Constant (k_a)

The absorption rate constant is a fractional rate of drug absorption from the site of administration into the systemic circulation. The rate of absorption determines the required time for the administered drug to reach an effective plasma concentration and may thus affect the onset of the drug effect. This rate influences both the peak plasma concentration (Cmax) and the time it takes to reach this peak (tmax).It has units of time^{-1} .

Elimination rate constant (k_{el})

The elimination rate constant is used to describe the rate at which a drug is removed from the body. It has units of time^{-1}. Rates of elimination can be separated as either zero-order or first order elimination kinetics.

- If the amount of a drug is decreasing at a constant rate then the elimination of the drug is a zero –order elimination .The plasma concentration – time profile during the elimination is linear and the elimination process is independent of

the concentration of the drug present in the body. It can be described as $\frac{dA}{dt}$ = - k_{el} . Zero order elimination is rather rare.

- If the amount of drug is decreasing at a rate that is proportional to amount, then the elimination is a first-order elimination and can be described as $\frac{dA}{dt}$ = - k_{el}x A. The plasma concentration – time profile during the elimination phase shows an exponential decrease. Most drugs used in clinical practice are eliminated in his manner.

Elimination half-life ($t_{1/2}$)

The time required to reduce the plasma concentration to one half its initial value is defined as the elimination half-life and described by the formula$t_{1/2}=\frac{\ln(2)}{k_{el}}$.

Clearance (CL)

Drug clearance is defined as the volume of plasma in the vascular compartment cleared of drug per unit time by the process of metabolite and excretion. Mathematically, clearance is the product of the first order elimination rate constant(k_{el})an the apparent volume of distribution (V).Thus,$CL = k_{el}$ x V. Hence the clearance is the elimination rate constant from the volume of distribution and is related to half-life by $t_{1/2}=\frac{\ln(2)\text{x V}}{CL}$. Clearance has a unit of Volume/time (e.g.L/h).

2.1.3 PHARMACOKINETIC MODELS

Pharmacokinetic models are hypothetical structures that are used to describe the fate of the drug in a biological system following its administration. In other words, these models describe the relationship between drug concentrations and time. In order to model the pharmacokinetics the term of "compartments" is introduced. Compartments are regions of the body in which the drug is well mixed and kinetically homogeneous. Compartments are the building blocks of many pharmacokinetic models. A description of the commonest compartment models is given below.

- One-compartment model

One-compartment model is the simplest form of compartment modeling, where the drug can enter and leave the body ("open" model) and the entire body is modeled as a kinetically homogeneous unit. The drug distributed instantaneously throughout the body and the drug equilibrates instantaneously between tissues. Thus, the drug log concentration-time profile shows a monophasic response, as presented in FIGURE 3. The model parameters are the absorption rate constant, the volume of distribution and the clearance. As we mentioned above, a re-parameterization of the model can be done if we use the elimination rate constant. In FIGURE 4, a one-compartment model is shown.

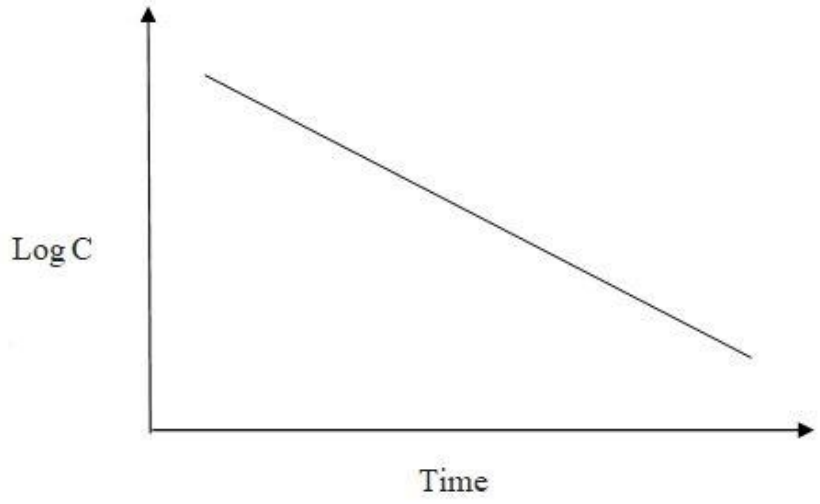


FIGURE 3. TIME PROFILE OF A ONE-COMPARTMENT MODEL SHOWING THE LOG CONCENTRATION (C) VERSUS TIME.

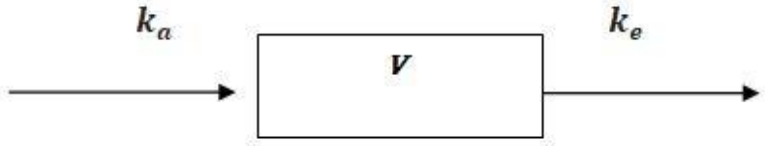


FIGURE 4. ONE-COMPARTMENT MODEL WITH ABSORPTION RATE CONSTANT, VOLUME OF DISTRIBUTION AND CLEARANCE.

The representation of a PK model can be done with algebraic or differential equations. Consider the simplest route of administration, a single intravenous bolus injection, of an initial dose D at time=0. The algebraic equation is:

$$C(t) = \frac{D}{V} e^{-\frac{CL}{V} * t} \quad \text{(eq. 1)}$$

The independent variable is time (t), and the dependent is the concentration. The notation C(t) indicates that C depends on t. Dose, clearance and volume of distribution are constant parameters; they do not change with different values of t.

Some complex systems cannot be stated as algebraic equations. A simpler way is to obtain the solution rewriting the (eq.1) as a differential equation:

$$\frac{dC}{dt} = -\frac{CL}{V} * C, \quad C(0) = \frac{D}{V}$$

Where $\frac{dC}{dt}$ is the rate of change of concentration with respect to time and the value of C at time =0 is C(0).

- Two-compartment model

Two–compartment model is an extension of one-compartment model. Two compartment models divide the body into a central and a peripheral compartment. The central compartment consists of tissues which are highly perfused, such as liver, heart, lungs, etc. and the peripheral compartment comprises less tissues where the distribution of the drug are slower, such as fat, muscle and skin. Following drug administration into the central compartment, the drug distributes between that compartment and the peripheral compartment. However, the drug does not achieve instantaneous distribution. Under these circumstances, the drug log concentration–time profile shows a biphasic response as shown in FIGURE 5.

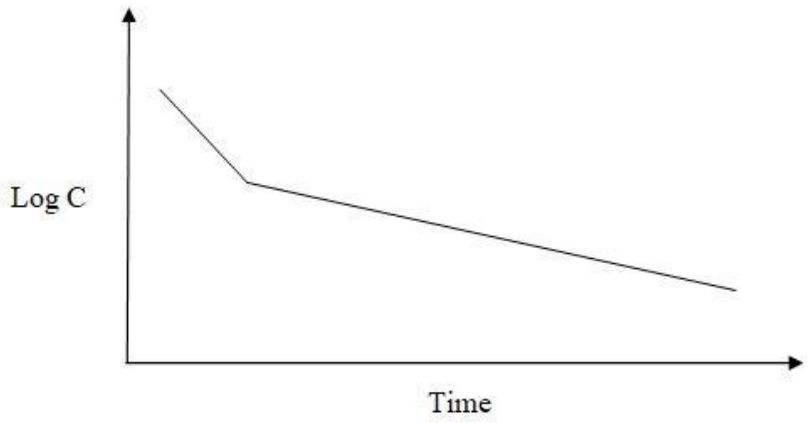


FIGURE 5. TIME PROFILE OF A TWO-COMPARTMENT MODEL SHOWING THE LOG CONCENTRATION (C) VERSUS TIME.

Moreover, it is important to understand that these compartments have no physiological meaning. A two compartmental model with first order absorption and elimination is illustrated in FIGURE 6. The parameters are used are; the volume of distribution in central compartment (V_c), the volume of distribution in peripheral compartment (V_{per}), the inter-compartmental rates constants (k_{12}= rate constant of transfer from central to peripheral compartment and k_{21} = rate constant of transfer from peripheral to central compartment),the absorption rate constant k_a and elimination rate constantk_{el} .

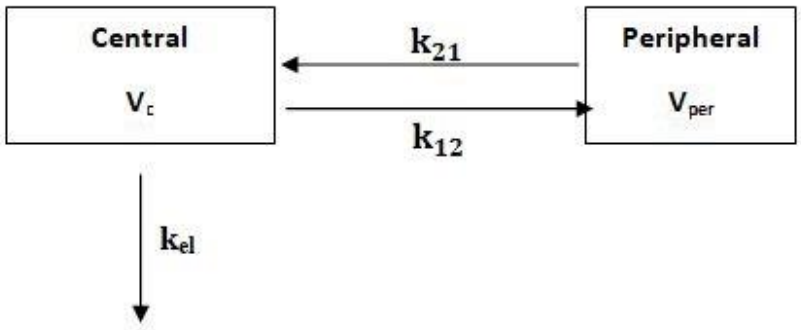


FIGURE 6. A TWO-COMPARTMENT MODEL WITH FIRST-ORDER ABSORPTION AND ELIMINATION WITH TWO VOLUMES OF DISTRIBUTION V_CAND V_{PER}, INTER COMPARTMENTAL RATES CONSTANTS k_{12}, k_{21} , THE ABSORPTION RATE CONSTANT k_a AND ELIMINATION RATE k_{el}

If we consider A_1 and A_2 as the amounts of drug in central compartment and peripheral compartment, respectively, the ordinary differential equations system, which describes the kinetics is:

$$dA_1/dt = -k_{12} * A_1 + k_{21}A_2 - k_{el} * A_1$$

$$dA_2/dt = k_{12} * A_1 - k_{21} * A_2$$

Dividing by volumes of distribution, we can obtain the concentration in each compartment. (Soraya Dhillon and Andrzej Kostrzewski 2006).

Furthermore, an extension to one and two-compartment models, is the multi-compartment models i.e. a three-compartment model, where the drug is distributed into more than one compartments. In addition to that, by using appropriate compartment models, we are able to describe the different properties of a drug such as different routes of administration and non-linearities in absorption or in elimination. In the present thesis, we are going to use another type of compartment model, known as transit compartment model. A detailed description is given below.

- Transit compartment model

It is not an unusual phenomenon that after an oral drug administration, some time passes before the drug appears in the systemic circulation. This phenomenon is known as an absorption delay. In order to deal with this matter, a lag time parameter (lag model) was used so as to describe the absorption delays. Nonetheless, Nerella et al.(1993),showed that lag time parameter can lead to incorrect estimates of pharmacokinetic parameters. An alternative method that has been proposed in order to assess the drug absorption is a multiple step process by introducing the transit compartmental model. Transit compartment absorption models are represented by a chain of pre-systemic compartments, without assigning a physical correlate to each transit compartment. In FIGURE 7, a schematic view of the drug flow through the chain of transit compartments is illustrated. According to the work of Savic et al. (2007), a comparison of the performance of lag model with the performance of the transit compartment model proved that by using the transit model resulted in a statistically significant improvement in the model fit compared to the lag model and in a better estimation of the absorption delay.

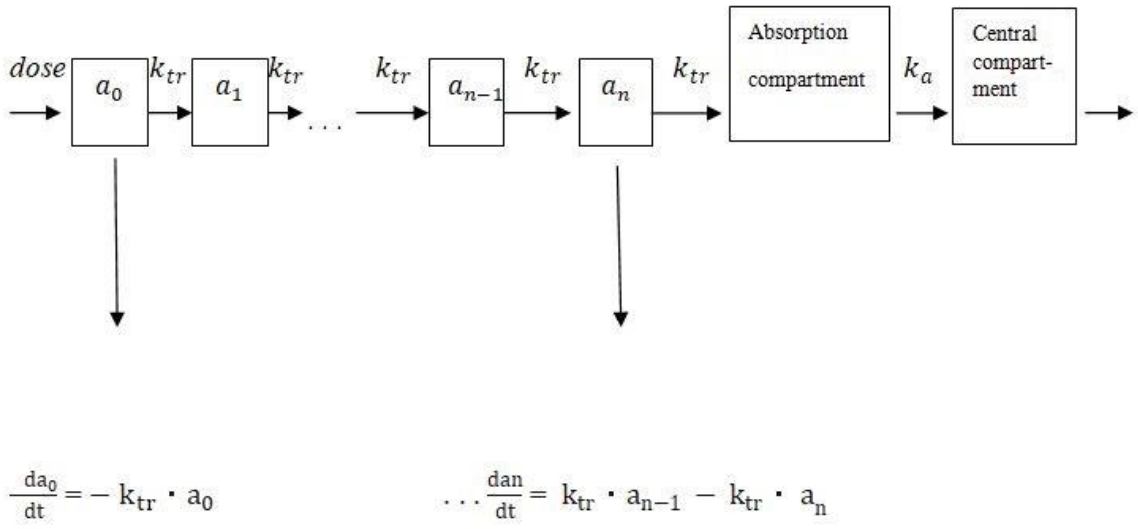


FIGURE 7. SCHEMATIC VIEW AND MATHEMATICAL DESCRIPTION OF THE DRUG FLOW THROUGH THE CHAIN OF TRANSIT COMPARTMENTS.

This model described the absorption delay by the passage of drug through a series of transit compartments with a single transfer rate constant, k_{tr}.
The rate of change of the amount of drug in the nth compartment is given by:

$$\frac{dan}{dt} = k_{tr} \cdot a_{n-1} - k_{tr} \cdot a_n$$

Where $\frac{dan}{dt}$ stands for the rate of change of substance a in compartment n at time t, a_n is the drug amount in the nth compartment at time t, k_{tr}stands for a transit rate constant from nth−1 compartment to the nth compartment and n is the number of transit compartments.

Following the administration, the drug is transferred through the series of transit compartments and from the last of the pre-systemic transit compartments to the central compartment via an absorption compartment in which the disappearance of drug was described with the rate constant k_a. The rate of change of drug amount in the absorption compartment $\frac{dAa}{dt}$ is given by:

$$\frac{dAa}{dt} = Dose \cdot F \cdot k_{tr} \cdot \frac{(k_{tr} \cdot t)^n \cdot e^{-k_{tr} \cdot t}}{\sqrt{2\pi} \cdot n^{n+0.5} \cdot e^{-n}} - k_a \cdot Aa$$

where F stands for drug bioavailability, Aa stand for the drug amount.

In this thesis we are going to use an additional useful parameter used in transit models. It is the mean transit time (MTT), which represents the average time spent by drug molecules traveling from the first transit compartment to the absorption compartment. The relationship between MTT, n and k_{tr} is shown in the equation below:

$$k_{tr} = \frac{n+1}{MTT}$$

2.2 POPULATION PHARMACOKINETIC MODELING

In the previous section an introduction to the field of pharmacokinetics is given. In this section the population pharmacokinetic modeling needed for this thesis is presented.

Modeling and simulation is an important tool in drug development. By implementing models we can describe and understand the time course of drug exposure and response, which follows the administration and we can provide a means for estimating the associated parameters e.g. the volume of distribution. Population pharmacokinetic models not only describe the above referred processes but also can investigate sources of variability in patient exposure. Population pharmacokinetics is the study of pharmacometrics at the population level, in which data from all individuals in a population are evaluated simultaneously using a nonlinear mixed effects model. (Mould and Upton, 2013)

2.2.1 NONLINEAR MIXED EFFECTS MODELS

Nonlinear mixed effects models are used to analyze population data. The term "nonlinear" refers to the fact that the dependent variable (e.g. concentration) is nonlinearly related to the independent variable (e.g. time). By the term "mixed effects" we are referring to the parameterization; mixed effects consist of fixed and random effects. Fixed effects are population parameters that they do not vary across individuals and random effects are random variables associated with each individual from the population. In order to build a population pharmacokinetic model, accurate information on dosing, covariates are also required.

Population pharmacokinetic models are comprised of three different components: structural models, stochastic models and covariate models. (Mould and Upton, 2012)
The structural models are functions that describe the time course of a measured response and can be representing as algebraic or differential equations. We have already given a description of structural models in section 2.1.3. Stochastic or statistical models describe the variability of the observed data and covariates models describe the influence of factors such as demographics or disease on the individual time course. These components are described in the below sections.

2.2.1.1. STATISTICAL MODELS

The statistical model describes the variability around the structure model. In contrast to linear regression models , where only one level of unexplained variability exists ,the residual unexplained variability (RUV), population models consist of two sources of unexplained variability .The first one is the residual variability common to standard linear regression, which counts the difference between a particular observation and model predicted value for this observation and the second source of variability is the between subject variability (BSV),which explains the variability between parameter values for a particular subject and the population value of parameters.
There is also another source of variability known as between-occasion variability (BOV), where a drug is administered on two or more occasions in each subject that might be separated by a sufficient interval for the underlying kinetics to vary between

occasions.(Mould and Upton, 2012) In the present thesis, only residual variability and between-subject variability were applied.

- Between-Subject Variability (BSV)

As we mentioned above, fixed effects parameters, usually symbolized as β, have the same value for every subject in the population. They are also known as population parameters. Random effects, represented by b, reflect the difference between an individual's parameter value and the population value. As regards the BSV's parameterization, b assumed to be normally or log-normally distributed across the population being evaluated, with a mean of 0 and variance ω^2.The different variances of b are reported in a "Ω matrix" . In cases, where the random effects are treated as correlated then co-variances are also reported in the Ω matrix. Pharmacokinetic data are often modeled as log-normally distributed.
For example, the parameter of CL and V for the i^{th} subject would be written as:

$$CL_i = \beta_1 \cdot \exp(b_{1i})$$
$$V_i = \beta_2 \cdot \exp(b_{2i})$$

Where CL_i, V_i are the individual values of CL and V of i^{th} subject; β_1 , β_2 are the population CL and V, respectively and $b_{1i,}$ b_{2i} are the deviation from the population CL and V for the i^{th} subject, respectively, b is normally distributed, $b \sim N(0, \omega^2)$.
The different variances and covariance of b are reported in a "Ω matrix"as shown below:

$$\Omega = \begin{bmatrix} \omega^2_{CL} & 0 \\ \omega^2_{CL,V} & \omega^2_V \end{bmatrix}$$

Where ω^2_{CL} is the variance of CL, ω^2_V is the variance of V and $\omega^2_{CL,V}$ is the covariance between CL and V.

- Residual Unexplained Variability (RUV)

The difference between the dependent-variable (e.g. concentration) symbolized by y and the corresponding individual specific model predictions (f), defines the unexplained error (ε):

$$y - f = \varepsilon$$

There are several forms of residual error models. (Owen and Fiedler-Kelly 2014). Here we describe the error models present at this thesis:
The additive error model, where the residual error may be expressed with a single variance that is not dependent upon other factors and error is just simply added to the prediction, is written as:

$$y = f + \varepsilon$$

The proportional or multiplicative error model is utilized when the magnitude of error varies with the magnitude of the prediction and is written as follows:

$$Y = f + f \cdot \varepsilon \qquad \text{or} \qquad y = f \cdot (1+\varepsilon)$$

The combined additive and proportional error model includes an additive and a proportional component. The additive component dominates the total combined error when the predicted concentrations are low, while the proportional component of the combined error is greater as predicted concentrations increase. The combined error is expressed as:

$$y = f + f \cdot \varepsilon_1 + \varepsilon_2$$

Where the ε_1 is the random variable associated with the proportional residual variability, while ε_2 is the additive portion of the residual variability.
The residual error is assumed to be normally distributed and centered round zero with variance σ^2,$\varepsilon \sim N(0, \sigma^2)$. Collectively, all the residual error components are referred

to as the residual variance or "Σ matrix", where Σ is a general covariance structure that may depend on response values.

2.2.1.2 COVARIATES MODELS

In population modeling, covariates play an important role due to the fact that drug exposures may vary significantly according to them. Population modeling develops quantitative relationships between covariates (such as age and weight) and parameters accounting for the explainable between subject variability by incorporating the influence of covariates on fixed effects. There are several functions which incorporate the covariates effects on population model e.g. the linear function. Covariates can also be introduced to the model centered or normalized to the mean value of database or to a reference value. Normalizing covariate values is generally preferred from centering, as centering can give negative parameter values and cause numerical difficulties in parameters estimation. (Mould and Upton, 2013)

Normalized weight covariate can be expressed by the following equation:

$$\mathrm{CLi} = \beta_{\mathrm{CL}} \cdot (\mathrm{Wi}/\mathrm{Wstd})$$

Allometric scaling is an empirical examination of the relationships between body function and body size (body weight). Allometric equations have proven useful for the extrapolation of animal data to determine pharmacokinetic parameters in man. It has been proposed that these equations are also applicable over the human size range including the pediatric population. (Knibbe CA. et. al 2005)

Anderson and Holford proposed the below mentioned allometric power model in order to scale metabolic processes such as drug CL and V ,as follows:

$$\mathrm{CLi} = \beta_{\mathrm{CL}} \cdot (\mathrm{Wi}/\mathrm{Wstd})^{0.75}$$
$$\mathrm{Vi} = \beta_{\mathrm{V}} \cdot (\mathrm{Wi}/\mathrm{Wstd})^{1}$$

where 0.75 is the empirically derived constant and exponent for clearance, CL_i is the clearance in the individual of weightWi,1 is the empirically derived constant and

exponent for the volume of distribution, β_{CL} is the clearance in a standardized individual with weight Wstd, Vi is the volume of distribution of weight Wi and β_V is the volume of distribution in a standardized individual with weight Wstd.

As regards the pediatric population, growth and development are two major aspects of children not readily apparent in adults. Clearance in the pediatric population should be investigated using models that describe size, maturation and organ function influences. Babies must grow from an immature form to reach a size that allows reproduction. This maturation factor cannot be explained by allometry. Consequently, allometry alone is insufficient to predict clearance in neonates and infants from adult estimate.

For this reason, in addition to allometric scaling, the rationale of introducing a maturation model was encouraged (Anderson and Holford, 2009). The equation which describes the maturation process is expressed as:

$$\mathrm{MF} = 1/[1 + (\mathrm{PMA}/\mathrm{TM}_{50})^{-\mathrm{Hill}}]$$

where MF is the maturation factor, TM_{50} describes the maturation half-time, or in other words is the age at which maturation reaches 50% of the final value, while the Hill coefficient relates to the slope of this maturation profile and PMA is referred to post-menstrual ageand is equal to the post-natal age plus the gestation time.

CHAPTER 3

3. MATHEMATICAL THEORY

The process of drug development is not an easy issue. Before the development of the new drug, clinical trials take place. A randomized clinical trial can take a long time and be expensive. Pharmaceutical companies spend a lot of effort, time and money in order to design a proper clinical trial using prior knowledge effort. If the assumptions are wrong, the trial may yield unsatisfactory results. If this happens, it is always too late for the trial to start from scratch; the sponsor's investment of time, money, and effort may have been wasted and patients have been subjected to unnecessary inconvenience, discomfort, and health risks.

For this reason, experimental design is used so as to prevent as far as possible these problems. Using prior knowledge from previous studies and patient characteristics, experimental design can provide useful information in order to improve the upcoming trials. This information could be defined as knowledge about the appropriate sampling points, sample size or even dose selection.

Some basic mathematical theory needed for this thesis is presented in this chapter.

3.1 FISHER INFORMATION MATRIX (FIM)

An efficient pharmacokinetic trial is that which can estimate the pharmacokinetic parameters with high precision. Design plays a vital role in order to obtain efficient parameter estimates. Methods based on Fisher Information Matrix are used in the field of population pharmacokinetic modeling in order to optimize designs. Many scientists have been worked to great extend with the development and implementation of population Fisher information matrix for non- linear mixed effects models. (Retout et al. 2001, Retout et al. 2002).

The general idea is that; the relationship between FIM and the variance-covariance matrix is based on Rao-Cramer inequality. The Rao-Cramer inequality states that the inverse of FIM is the lower bound of the variance-covariance matrix of any unbiased estimator of the population parameters.

To derive the FIM, we first need to specify the non-linear mixed effects models that describe the pharmacokinetic of the drugs. The population model is described by the non-linear mixed effects structure. It is assumed that:

$$y_i = f(\theta_i, \xi_i) + \varepsilon_i$$

Where y_i is the n_i–vector of observations for the i^{th} individual, f is a function describing the nonlinear model, $\xi_i = (t_{i1}, t_{i2}, \dots, t_{in_i})^T$ is the n_i–vector of sampling times of i^{th} individual and θ_i is the p-vector of individual parameters and ε_i is the n_i–vector of random effects with $\varepsilon_i \sim N(0, \Sigma_i)$, Σ_i are assumed to be n_i x n_i diagonal matrices. Let b_i to symbolize the p-vector of random effects and β the p-vector of fixed effects. It is assumed, as usual, that $b_i \sim N(0, \Omega)$, with Ω defined as p x p-diagonal matrix with ω_k^2 representing the variance of k^{th} component of random vector. We assume $\varepsilon_i | b_i$ to be independent from one subject to the other and for each subject ε_i and b_i are also independent.

The expression of inter-individual variability is given by exponential form as $\theta i = \beta \cdot \exp(b_i)$. Consequently, $f(\theta_i, \xi_i)$ can be also noted as $f(\beta, b_i, \xi_i)$.

Finally, we note as Ψ the vector of all population parameters to be estimated, so that $\Psi^T = (\beta^T, \omega_1^2, \dots, \omega_k^2, \sigma_{add}^2, \sigma_{prop}^2)$

3.1.1 THE ELEMENTARY FISHER INFORMATION MATRIX

The elementary Fisher Information Matrix, for only one individual with design ξ is given by the form:

$$M_F(\Psi, \xi) = E\left(-\frac{\partial^2 l(\Psi; y)}{\partial \Psi \, \partial \Psi^T}\right) \quad \text{(eq. 2)}$$

Where $l(\Psi; y)$ is the log likelihood of the vector of observation y of the individual for the population parameters Ψ. The notation $M_F(\Psi, \xi)$ is used to stress the fact that information matrix depends on the underlying design ξ and population parameters Ψ and operator E() denotes the expectation. The subscript i is omitted, at this section,

for simplicity. Because of the non-linearity of the structural model f with respect to parameters θ, there is no analytical expression for l(Ψ; y); Retout et al. (2002) proposed a development of the M_F (Ψ, ξ), by using the first-order Taylor expansion of the structural model f(β ,b,ξ), around the expectation of b ,that is around 0.The statistical model can thus be written

$$y= f(\beta ,b,\xi) + \varepsilon \cong f(\beta, 0, \xi) + \frac{\partial^T f(\beta,0,\xi)}{\partial b} b + \varepsilon$$

and then

$$E(y) \cong f(\beta, 0, \xi)$$

$$Var(y) = V \cong \frac{\partial^T f(\beta,0,\xi)}{\partial b} \Omega \frac{\partial f(\beta,0,\xi)}{\partial b} + \Sigma$$

Where Σ are n x n-diagonal matrix, Ω is p x p-diagonal matrix and the $\frac{\partial^T f(\beta,0,\xi)}{\partial b}$ is a n x p- matrix. Since b and ε are assumed be normal the log-likelihood l is then approximated by $l(\Psi;y) \cong -\frac{1}{2}(n\ln(2\pi) + \ln|V| + \left(y - f(\beta, 0, \xi)\right)^T V^{-1}(y - f(\beta, 0, \xi)))$

Consequently, the elementary fisher information matrix is approximated by a block-diagonal matrix and is rewritten to the form:

$$M_F(\Psi, \xi) = \frac{1}{2}\begin{bmatrix} A(E,V) & 0 \\ 0 & B(E,V) \end{bmatrix}$$

Where A is the matrix of fixed effects and B the matrix of random effects.

3.1.2 THE POPULATION FISHER INFORMATION MATRIX

For more than one individual, the elementary Fisher information matrix is developed to population Fisher Information Matrix. For a population design $\Xi=\{\xi_{1,\dots},\xi_N\}$, the sum of the N elementary Fisher information matrices $M_F(\Psi, \xi_i)$, for each subject i with design ξ_i of the study is :

$$M_F(\Psi, \xi) = \sum_{i=1}^{N} M_F(\Psi, \xi i)$$

The population fisher information matrix is usually composed of a limited number of Q elementary designs, each one of them is composed of a set of n_q sampling times and it is performed in a number of $N_q$of subjects. This is expressed by:

$$M_F(\Psi, \xi) = \sum_{q=1}^{Q} N_q x M_F(\Psi, \xi_q)$$

The expected values of the standard errors for each population parameter are computed as the square root of the diagonal elements of the inverse of $M_F(\Psi, \xi)$; these values are from Cramer Rao inequality, the lower bound of the standard errors of parameter estimation.

3.2 D-OPTIMAL DESIGN

D-optimality is the most common tool in optimal design. For a given vector $\Psi_0^T = (\beta^T, \omega_1^2, \dots, \omega_k^2, \sigma_{add}^2, \sigma_{prop}^2)$ of population parameters, a population design Ξ is D-optimal if it maximizes the determinant of the Fisher information matrix :

$$\Xi = \arg\max_{\Xi} |M_F(\Psi_0^T, \Xi)|$$

An optimization algorithm is needed to be applied in order to optimize the sampling design by maximizing the determinant of the Fisher information matrix. In the present thesis, particle swarm optimization (PSO) algorithm was used for optimization procedure.

A concept for the optimization of nonlinear functions using particle swarm methodology is introduced in 1955 by Kennedy and Eberhart. The Particle swarm optimization algorithm was first intended for simulating social behavior, as it is inspired from the nature social behavior and dynamic movements with communications of insects, birds and fish. The main concept of the algorithm is that; it uses a number of agents (particles) that constitute a population (swarm) moving around in the search space looking for the best solution. Each particle in search space adjusts its "flying" according to its own flying experience as well as the flying experience of other particles.(Kennedy and Eberhart, 1995)

Consider an objective function f: $R^n \rightarrow$ R. To minimize the function f, we should find an a $\in$ R so that: $\forall$ b $\in R^n$: f(a)$\leq$ f(b). Then, a is called a global minimum for the function f. It is usually not possible to pinpoint the global minimum exactly in optimization and candidate solutions with sufficiently good fitness are deemed acceptable for practical reasons. In PSO the candidate solutions are the particles. The particle swarm algorithm begins by creating the initial particles, and assigning them initial velocities. It evaluates the objective function at each particle location, and determines the best (lowest) function value and the best location. It chooses new velocities, based on the current velocity, the particles' individual best locations, and the best locations of their neighbors. It then iteratively updates the particle locations (the new location is the old one plus the velocity, modified to keep particles within bounds), velocities, and neighbors. The termination criterion can be the number of iterations performed, or a solution where the adequate objective function value is found. Some advantages of the PSO algorithm are; the simple implementation of the algorithm, it needs very few algorithm parameters and it is a very efficient global search algorithm.(Pederson, 2010)

3.3 EFFICIENCY CRITERION

The determinant of Fisher information matrix has one more important use. A criterion Φ, known as efficiency criterion, is defined as the determinant standardized by the dimension of the vector Ψ;

$$\Phi(\Xi) = |M_F(\Psi, \Xi)|^{1/\dim(\Psi)}$$

and has the ability of comparing the efficiency between the several designs. Designs can be compared by the evaluation of the criterion Φ. The efficiency of a population design $\Xi_1$with respect to a population design Ξ_2 is given by $\Phi(\Xi_1) / \Phi(\Xi_2)$. If the population design Ξ_1 is more efficient than Ξ_2, this ratio will be greater than 1. (Retout et al. 2001)

3.4 MODEL EVALUATION

"Model evaluation" is an important feature in model validation procedure as it required for both processes; to diagnose one or several intermediary or key models in a model-building procedure or evaluate a selected model with respect to the modeling objectives.

NONMEM (non-linear mixed effects models) is the first software available for population PK modeling. (Owen and Fiedler-Kelly 2014).This software is a model analysis program that can be used to fit models to many types of data. Three model evaluation techniques are described below.

- Goodness of Fit plots

Although there are many statistical tools for model evaluation, the primary tool for most biomedical science is graphical evaluations. Graphical methods have an advantage over numeric methods for model evaluation because they readily shed light on a broad range of complex aspects of the relationship between the model and the data. The fundamental diagnostic plot for the model evaluation in pharmacokinetic modeling is the scatter plot of individual predicted concentrations (IPRED) versus the

observed concentrations (DV).This plot compares the measured values of concentrations of the drug with the corresponding individual- specific predicted values. It represents the goodness of fit of the model after accounting for the subject specific random effects terms.

- Visual Predictive Check Plots (VPC)

The Visual Predictive Check (VPC) plot is a popular tool for evaluating the performance of population PK models. The principle of the VPC is to assess graphically whether simulations from a model are able to reproduce both the central trend and variability in the observed data, when plotted versus an independent variable (usually time). In other words, a VPC will diagnose both the fixed and random effects in a mixed-effects model .VPCs generally involve simulation of data from the original or new database. The final model is used to simulate new data sets using the selected database design, and prediction intervals (usually 95%) are constructed from simulated concentration time profiles and compared with observed data. Percentiles of the simulated data are compared to the corresponding percentiles of the observed data. The percentiles are calculated either for each unique value of the independent variable (usually time) or for a bin (interval) across the independent variable. Data binning or grouping of simulated observations within small intervals of time following dosing is often performed to prevent a very erratic –looking profile. (Joel S. Owen and Jill Fiedler-Kelly, 2014)Typically, the median, the 2.5th and the 97.5th percentiles are presented. If the model is correct, the observed percentiles should be close to the predicted percentiles and remain within the corresponding CI. (Nguyen et al. 2017)

- Bootstrap Method

In non-linear mixed effects models the uncertainty of the parameters is usually quantified by the standard errors (SE) obtained asymptotically by the inverse of the Fisher information matrix (MF) and by the asymptotic confidence intervals (CI) which are assumed to be normal and symmetric. However, this uncertainty might be biased when the assumption of asymptotic normality for parameter estimates and their SE is incorrect. Sometimes, they cannot be even obtained due to the over-parameterization of the model or numerical problems when evaluating the inverse of the MF. (Thai HT et al. 2014)

For this reason, besides the graphical tools, numeric methods, are always used in parallel to provide additional information for the reliability of a model. Re-sampling based methods, such as bootstrap, are some of them. Bootstrap methods are re-sampling techniques that provide an alternative for estimating parameter precision. They are useful to verify the robustness of standard approximations for parameter uncertainty in parametric models. Bootstrap is a robust method as it assess the uncertainty of parameters while avoids parametric assumptions made when computing CIs using other methods. The principle idea of bootstrap is that; it generates replicate data sets of the same size as the original dataset where individuals are randomly drawn from the original database and can be drawn multiple times or not drawn for each replicate. In order to adequately reflect the parameter distributions, many replicates (at least 500) are generated and evaluated using the final model, and replicate parameter estimates are tabulated. The percentile bootstrap CI are constructed by taking the lower 2.5% and the upper 97.5% value of each parameter estimate.For most pharmacokinetic databases, <30% SE for fixed effects and <50% SE for random effects are usually achievable (generally, the SE for random effects are higher than the SE for fixed effects). (Mould and Upton, 2013)

CHAPTER 4

4. PRESENT STUDY

4.1 BACKGROUND AND OBJECTIVES

Often in pediatric drug development, pharmacokinetic studies are employed as part of an adults-to-children extrapolation plan. However, even pharmacokinetic studies present challenging ethical limitations. Characterizing pharmacokinetic is optimally done by the use of mathematical modeling which is ideal for pediatrics as it allows sparse sampling. The scope of the present work is the application of D-optimal design for the design of a pharmacokinetic pediatric trial to study a fixed dose combination product of isoniazid, rifampicin and pyrazinamide with sparse sampling, for the treatment of tuberculosis.

4.2 METHODS

A priori knowledge

In the present thesis, three pharmacokinetic models and their initial pharmacokinetic parameters, were assumed as a priori knowledge. This a priori knowledge, that we used in order to find the optimal blood sampling time points for the three dugs simultaneously, were derived from the analysis of published data from previous studies. Below we briefly describe the subjects, patients' therapy and the sampling time that these data were produced.

Zvada et al.(2014) present at their work the demographic and clinical characteristics from the pediatric population. This work used combined data of 76 South African children with tuberculosis. The children were separated into two cohorts. The first cohort included 56 children and the second cohort had 20 children.

Daily doses of rifampicin and isoniazid were given for 6 months with pyrazinamide added for the first 2 months. Dispersible tablets formulated for children were used. In Cohort 1 median daily doses of rifampicin, pyrazinamide and isoniazid approximated 10, 23 and 5 mg/kg, respectively. In Cohort 2, two pharmacokinetic occasions were

carried out. In the first occasion median daily doses were adjusted as 10, 25 and 5 mg/kg and in the second occasion 15, 36 and 10mg/kg were given for rifampicin, pyrazinamide and isoniazid, respectively.

As regards the blood sampling, the blood sampling for Cohort 1 was conducted in the first and fourth month after starting treatment, at 0.75, 1.5, 3, 4, 6 h after dosing. The blood sampling for Cohort 2 was conducted at some time after the two weeks after starting treatment and the second blood sampling was repeated one week later, at pre-dose, 0.5, 1.5,3 and 5 h post-dose.

Zvada et al. concluded in the following pharmacokinetic models for the analysis of the concentration-time data that acquired from the children population. Our aim was to use these pharmacokinetic models and their parameters estimates as initial values for our analysis. For every dug, a full description of the respective pharmacokinetic model, is presented in the next sections.

Rifampicin Model

The pharmacokinetic model that we used for rifampicin, is one-compartment model with transit absorption compartments and first order elimination. The differential equation system that describes the pharmacokinetic model of rifampicin is:

$$\frac{dA1(t)}{dt} = D \cdot F_{rel} \cdot k_a \cdot \frac{(k_a \cdot t)^{n+1} \cdot e^{-k_a \cdot t}}{\sqrt{2\pi} \cdot (n+1)^{n+1.5} \cdot e^{-n-1}} - \frac{CL}{V} \cdot A_1(t), \quad A_1(0)=0 ,$$

$$k_{tr}=k_a=\frac{n+1}{MTT} \qquad \text{and}$$

$$C(t)=\frac{A_1(t)}{V}$$

Where $\frac{dA1}{dt}$ is the rate of change of the amountof drug in the central compartment at time t, A_1 is the drug amount in the central compartment; C the concentration at time t, D being the dose; CL, the clearance; V, the volume of distribution ; k_a, the first

order absorption rate constant; MTT, the absorption mean transit time (value at full maturation) ; k_{tr}, the transit rate constant ;n ,the number of transit compartments ; F_{rel} ,the relative bioavailabilty ;

The model has a combined variability of an additive and proportional error terms. There is also between subject variability on parameters of clearance and volume of distribution. We also include a BSV parameter on the fixed effect of absorption mean transit time parameter, in order to have a more informative model as far as the variability in the absorption phase is concerned. The random effects are assumed to be of exponential form.

An allometric weight model was applied to standardize the pharmacokinetic parameters using a standard weight of 12.5kg.The allometric weight model for clearance is given by $(\mathrm{wt}/12.5)^{0.75}$ and for volume parameter is given by $(\mathrm{wt}/12.5)^{1}$ The equation for clearance and volume of distribution is:

$$\mathrm{CL_i}=\beta_{\mathrm{CL}} \cdot \mathrm{MF} \cdot (\mathrm{wt_i}/12.5)^{0.75} \cdot \exp(\mathrm{b_{CLi}}), \qquad \mathrm{b_{CLi}} \sim \mathrm{N}(0, \omega^2_{\mathrm{CL}})$$

With $\mathrm{MF}= 1/ [1+(\mathrm{PMA}/\mathrm{TM_{50}})^{-\mathrm{Hill}}]$ and

$$\mathrm{V_i}=\beta_{\mathrm{V}} \cdot (\mathrm{wt_i}/12.5)^{1} \cdot \exp(\mathrm{b_{Vi}}) \quad , \quad \mathrm{b_{Vi}} \sim \mathrm{N}(0, \omega^2_{\mathrm{V}})$$

Where $\mathrm{CL_i}, \mathrm{V_i}$ are the scaled typical value of CL and V for individual i, respectively;β_{CL}, β_{V} are the population estimates for CL and V, respectively; $\mathrm{b_{CLi}}$,$\mathrm{b_{Vi}}$ are the deviation from the population CL and V, respectively; ω^2_{CL},ω^2_{V} are the variances of CL and V, respectively; $\mathrm{wt_i}$ is the body weight of individual i in kg; MF is the maturation factor; $\mathrm{TM_{50}}$ is the post-menstrual age at which 50% of clearance and mean transit time maturation is achieved; Hill is the steepness of the maturation function; PMA is the age derived by adding 36 weeks to the post-natal age, assuming no premature birth.

The initial parameters values, used in our analysis, are presented in TABLE 3.

TABLE 3.INITIAL PARAMETERS VALUES OF RIFAMPICIN MODEL

Parameters(units)	Initial Parameter Values
β_{CL}(L/h)	8.15
β_{V}(L)	16.2
β_{MTT}(h)	1.04
β_{n}	8.04
ω^2_{CL}	0.09
ω^2_{V}	0.16
ω^2_{MTT}	0.04
$\sigma^2_{add}(mg/L)^2$	0.01
σ^2_{prop}	0.04

Where β_{CL} , β_{V} , β_{MTT}, β_{n} are population estimates parameters of clearance, volume, mean transit time and number of transit compartments respectively; ω^2_{CL},ω^2_{V},ω^2_{MTT}, are inter-individual variances of clearance, volume and mean transit time, respectively and σ^2_{add} , σ^2_{prop} are the residual variances of additive and proportional error, respectively.

As described in published data (Zvada et al.2014), covariates such as HIV status and albumin levels had no influence on pharmacokinetics of rifampicin, so we do not incorporate them to the model.

TM_{50} and Hill parameters were considered as covariates and, according to published data, a fixed value equal to 58.2(weeks) and 2.21 was given, respectively.

Pyrazinamide model

The pharmacokinetic model that we used for pyrazinamide, is one-compartment distribution model with transit absorption compartments, first-order absorption and elimination, as described by the following differential equation system'

$$\frac{dA1(t)}{dt} = D \cdot F_{rel} \cdot k_{tr} \cdot \frac{(k_{tr} \cdot t)^n \cdot e^{-k_{tr} \cdot t}}{\sqrt{2\pi} \cdot (n)^{n+0.5} \cdot e^{-n}} - k_a \cdot A_1(t), \quad A_1(0)=0$$

$$\frac{dA2(t)}{dt} = k_a \cdot A_1(t) - \frac{CL}{V} \cdot A_2(t), \quad A_2(0)=0 \quad , \quad k_{tr} = \frac{n+1}{MTT} \quad \text{and}$$

$$C(t) = \frac{A2(t)}{V}$$

Where $\frac{dA1(t)}{dt}$ and $\frac{dA2(t)}{dt}$ are the rates of change of the amount of drug in the absorption and central compartment at time t, respectively; $A_1(t)$ and $A_2(t)$ are the drug amounts in the absorption and central compartment at time t; C the concentration at time t, D being the dose; CL, the clearance; V, the volume of distribution ; k_a, the first order absorption rate constant; MTT, the absorption mean transit time (value at full maturation); k_{tr}, the transit rate constant ; n, the number of transit compartments ; F_{rel} the relative bioavailabilty ;

The residual error of the model, presented in the published data, was proportional' consisted of two proportional error terms (10% proportional error for Cohort 1 and 6% proportional error for Cohort 2).In our analysis, a 10% proportional error was used as we wanted to examine the worst case scenario of residual variability. We also include an additive error term. Consequently, a combined error model was applied to the pharmacokinetic model of pyrazinamide.

With respect to the between subject variability of parameters, only the IIV of clearance was presented in the work of Zvada et al. (2014) For this reason, in order to have a more informative model about the inter-subject variability, we also include IIV on the below mentioned parameters' volume of distribution parameter, absorption rate

constant and absorption mean transit time. The initial parameters values of pyrazinamide's model, are presented in TABLE 4.

From the published data, no significant covariate relationship was supported and no maturation function was applied to the clearance parameter.

We applied to pyrazinamide's model, the same allometric weight model was applied to the parameters of clearance and volume of distribution as described in the rifampicin model.

TABLE 4. INITIAL PARAMETER VALUES OF PYRAZINAMIDE MODEL

Parameters(units)	**Initial Parameter Values**
β_{CL}(L/h)	1.08
β_{V}(L)	9.64
$\beta_{ka}(h)^{-1}$	4.48
β_{MTT}(h)	0.10
β_{n}	3.94
ω^2_{CL}	0.09
ω^2_{V}	0.09
ω^2_{ka}	0.09
ω^2_{MTT}	0.09
$\sigma^2_{add}(mg/L)^2$	0.01
σ^2_{prop}	0.01

Where $\beta_{CL}, \beta_{V}, \beta_{ka}, \beta_{MTT}, \beta_{n}$ are population estimates parameters of clearance, volume of distribution, absorption rate constant, absorption mean transit time and number of transit compartments, respectively; ω^2_{CL},ω^2_{v},ω^2_{ka} ω^2_{MTT}, are inter-individual variances of clearance, volume of distribution, absorption rate constant and mean

transit time, respectively and $\sigma^2_{add}, \sigma^2_{prop}$ are the residual variances of additive and proportional error, respectively.

Isoniazid model

The pharmacokinetic model that we used for isoniazid is a two-compartment distribution model with absorption transit compartments and first-order elimination. The differential equation system which describes the isoniazid model is shown below.The first equation describes the absorption compartment, the second equation, the central compartment and the third equation, the peripheral compartment.

$$\frac{dA1(t)}{dt} = D \cdot F_{rel} \cdot k_{tr} \cdot \frac{(k_{tr} \cdot t)^n \cdot e^{-k_{tr} \cdot t}}{\sqrt{2\pi} \cdot (n)^{n+0.5} \cdot e^{-n}} - k_a \cdot A_1(t), \; A_1(0)=0$$

$$\frac{dA2(t)}{dt} = k_a \cdot A_1(t) - k_{12} \cdot A_2(t) + k_{21} \cdot A_3(t) - \frac{CL}{V} \cdot A_2(t), \; A_2(0)=0$$

$$\frac{dA3(t)}{dt} = k_{12} \cdot A_2(t) - k_{21} \cdot A_3(t), \; A_3(0)=0$$

and

$$C(t)=\frac{A_2(t)}{V}, \; k_{12}=\frac{Q}{V}, \; k_{21}=k_{12}\frac{V}{Vper}, \; k_{tr}=\frac{n+1}{MTT}$$

Where $\frac{dA1(t)}{dt}$, $\frac{dA2(t)}{dt}$ and $\frac{dA3(t)}{dt}$ are the rates of change of the amount of isoniazid in the absorption, central and peripheral compartment at time t, respectively; $A_1(t), A_2(t)$ and $A_3(t)$ are the drug amounts at the absorption, central and peripheral compartment at time t ; C the concentration at time t; D is the dose; CL, the clearance; Q, the inter-compartmental clearance; V, the volume of distribution on central compartment; Vper, the volume of distribution on the peripheral compartment; k_a, the first order absorption rate constant; k_{12}, rate of transfer from central to peripheral compartment; k_{21} ,rate of transfer from peripheral to central compartment;MTT, the absorption mean transit time (value at full maturation); k_{tr}, the transit rateconstant;n,the number

of transit compartments and F_{rel} ,the relative bioavailability between different acetylator groups (see further down).

As in the residual error model of pyrazinamide, the proportional error of isoniazid was estimated separately for the two cohorts. The proportional error for Cohort 1 was 20% and for Cohort 2 was 7%. In our analysis, a 20% proportional error was assumed as we wanted to examine the worst case scenario of residual variability.

Similarly to pyrazinamide model, inter-subject variability was assumed for V, k_a, MTT parameters, as no available estimated values of these parameters could be obtained from the published data. No inter-individual variability was assumed for Q and Vper. Initial parameters values of isoniazid model are displayed in Table 5.
The same allometric weight model was applied to the parameters of clearance and volume, as described in rifampicin model. In case of isoniazid this scaling was applied on the inter-compartmental clearance and on the peripheral volume of distribution, too. Maturation function was also included in the equation of clearance.
NAT2 genotype was a significant covariate for the parameters of clearance and bioavailability. Clearance and bioavailability values differ according to the category of acetylatorfactor(slow, intermediate and fast). Acetylator factor was included into the equation of clearance as covariate.
The equations for clearance, volume of distribution, inter-compartmental clearance and on the peripheral volume of distribution are:

$$CL_{ij}=\beta_{CL} \cdot MF \cdot ACET_j \cdot (wt_i/12.5)^{0.75} \cdot \exp(b_{CLi}) \quad b_{CLi} \sim N(0, \omega_{CL}^2)$$

With $MF= 1/ [1+(PMA/TM_{50})^{-Hill}]$ and

$$V_i=\beta_V \cdot (wt_i/12.5)^1 \cdot \exp(b_{Vi}) \quad b_{Vi} \sim N(0, \omega_V^2)$$

$$Q_i =\beta_Q \cdot (wt_i/12.5)^{0.75}$$

$$Vper_i = \beta_{Vper} \cdot (wt_i/12.5)^1$$

WhereCL_{ij} is the scaled typical value of CL for individual i for j acetylator category (j=1,2,3 where 1=slow ,2=intermediate ,3=fast) , V_i, Q_i , $Vper_i$ are the scaled typical value of V, Q , Vper for individual i, respectively; β_{CL}, β_V , β_Q, β_{Vper} are the population estimates for CL, V,Q and Vper respectively; b_{CLi} ,b_{Vi} are the deviation from the population CL and V, respectively; ω^2_{CL},ω^2_V are the variances of CL and V, respectively;wt_i is the body weight of individual i in kg; MF is the maturation factor; TM_{50} is the post-menstrual age at which 50% of clearance and mean transit time maturation is achieved; Hill is the steepness of the maturation function; PMA is the age derived by adding 36 weeks to the post-natal age, assuming no premature birth.

As regards the number of compartments, i.e. the n parameter, a fixed value equal to 4 is provided by the data, as no estimation of this parameter is given.TM_{50} and Hill, were handled, as in rifampicin model, with fixed values equal to 49.0 (in weeks) and 2.19, respectively.

TABLE 5. INITIAL PARAMETER VALUES OF ISONIAZID MODEL

Parameters(units)	**Initial Parameter Values**
$\beta_{CL}(L/h)$	4.44
$\beta_{V}(L)$	11.0
$\beta_{ka}(h)^{-1}$	2.47
$\beta_{MTT}(h)$	0.179
β_{Q}	2.00
β_{Vper}	5.03
ω_{CL}^2	0.09
ω_{V}^2	0.09
ω_{ka}^2	0.25
ω_{MTT}^2	0.09
σ_{prop}^2	0.04

Covariates

The aim of this thesis was the design of a sparse sampling clinical trial in children with tuberculosis, mainly focused on the age ranged from 1 month to approximately 7.5 years old. The covariates used in the present thesis are age, weight and genotype.

I. Age and Weight

To start with, age and weight are important covariates that should be included in pediatric pharmacokinetic models. Although these covariates have an effect on pharmacokinetic parameters and consequently on sampling points, we handled them by averaging them out. By this way, a good comprise of the entire range of the covariates is achieved.

A separation of four different age groups was carried out. The first age group ranges from 1 month to 6 month with a step of a month, the second group from 7 months to 24 months with a step of 3months, the third from 25 to 45 with a step of 3 months and the fourth from 46 to 88 months with a step of 6 months. The step in every age group was decided in such way that the same amount of information of every group can finally be obtained. Consequently, 27 age values were generated, created an age distribution.

As regards the covariate of weight, the median weight (in kg) per age (in weeks) that we used in our analysis, was derived from WHOs' child growth standards tables. In FIGURE 8. - 9., the weight-for-age percentiles for boys from birth to 5 years and 5 to 10 years is presenting, respectively. As a consequence, four weight bands were created. The first weight band ranges from 5kg to 7.9kg , the second from 8kg to 11.9kg, the third from 12 kg to 15.9 kg and the last from 16 kg to 24 kg. Finally, 27 weight values were created, allocated to the above referred 27 age values.

Furthermore, we applied the recommended specifications of WHO (i.e. 75/50/150mg of rifampicin/isoniazid/pyrazinamide in each fixed dose combination tablet). The number of daily tablets used for the analysis were applied according to the above referred weight bands. More specifically, one tablet of FDC drug for children weighting 5-7.9 kg, two tablets for children weighting 8-11.9 kg, three tablets for children weighting 12-15.9 kg and four tablets for children weighting 16-24kg.

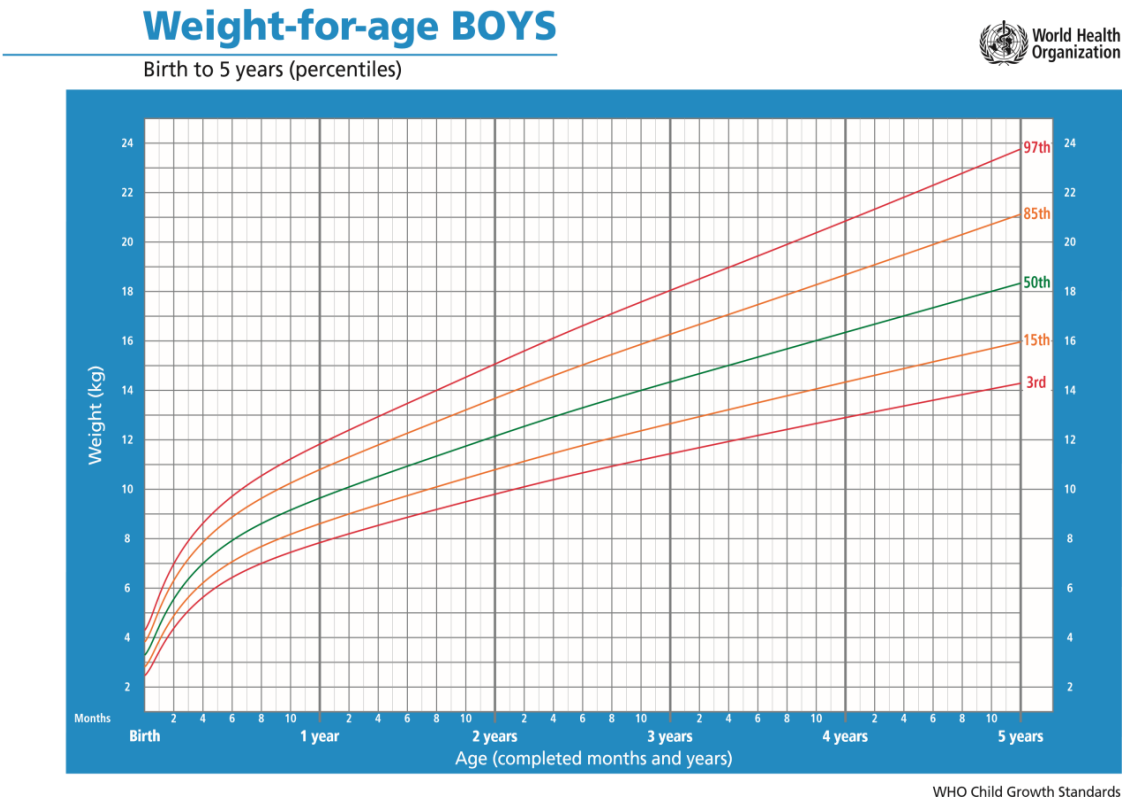


FIGURE 8. THE WEIGHT-FOR-AGE PERCENTILES FOR BOYS FROM BIRTH TO 5 YEARS.

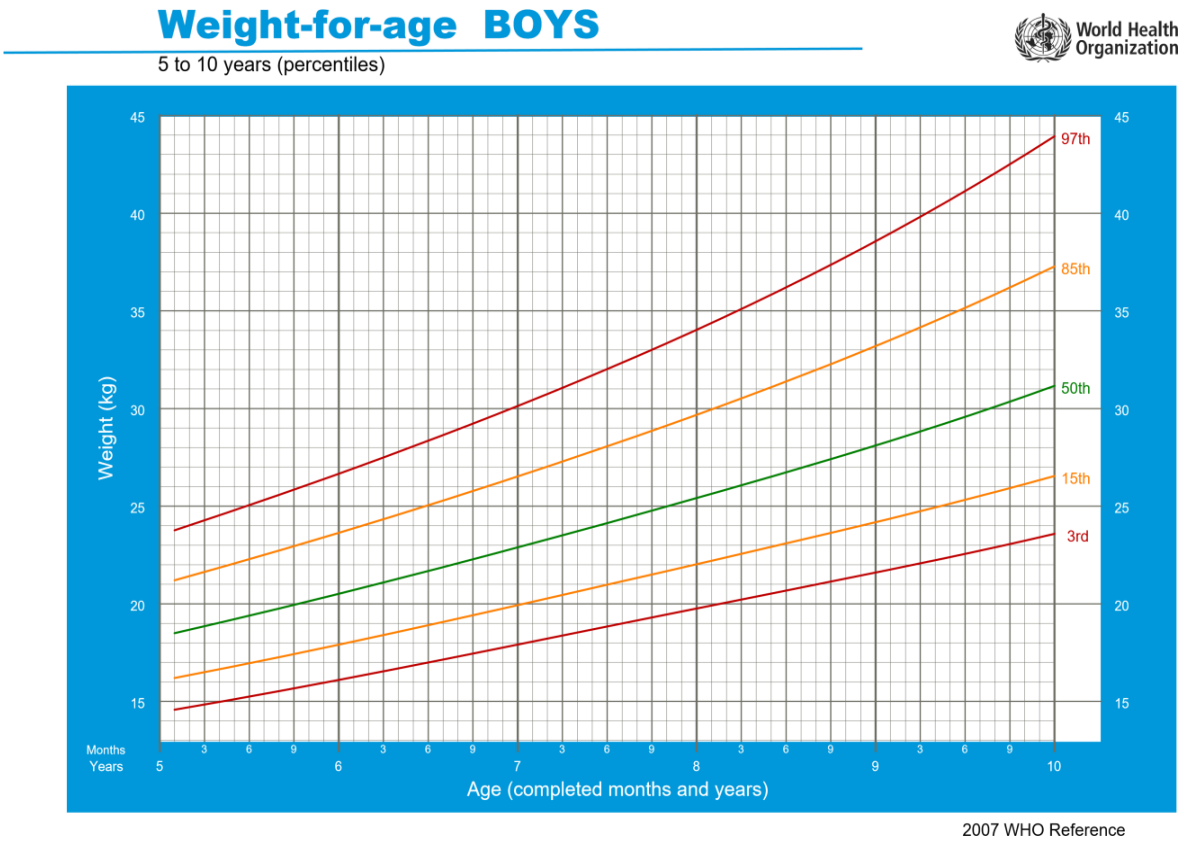


FIGURE 9. THE WEIGHT-FOR-AGE PERCENTILES FOR BOYS FROM 5 TO 10 YEARS.

II. NAT2 enzyme

Isoniazid differs from rifampicin and pyrazinamide as far as the metabolism is concerned. The reason is that the primary step in the metabolism of isoniazid is acetylation. Acetylation catalyzed by the enzyme, N-acetyltransferase (NAT2), resultsto the formation of acetyl INH. NAT2 enzyme displays genetic polymorphism. Human subjects show a wide degree of variation in their capacity to acetylate INH to acetyl INH. Individuals can be distinctly characterized phenotypically as being either slow or rapid acetylators (the activity of the enzyme being higher in rapid acetylators). Molecular techniques that are now available permit identification of three genotypes: rapid, intermediate and slow. Slow acetylators are known to be at a risk for most drug induced toxicities, while rapid acetylators are likely to experience decreased therapeutic efficacy. It has been suggested that NAT2 genotyping before therapy could be useful to predict adverse reactions and make dose adjustments, if necessary. The acetylator gene frequency for the slow allele differs widely across ethnic groups and countries: 10 per cent in people from the mongoloid race such as the Eskimos, Japanese and Chinese, 90 per cent in the Middle East, 60 per cent in the Negroid and Caucasian populations and 72 per cent in the USA. (Hemanth Kumar AK et al. 2017).

As in the present thesis, we used published data from previous conducted studies, we had to handle the covariate of NAT2 genotype. Oral clearance values of isoniazid differ according to the category of NAT2 enzyme. The categories of acetylators are separated into three groups, the slow, intermediate and fast acetylator category.
In order to handle this covariate, a simulation of data sample (27 values of genotyping were creating) was carried out. Weighting factors were used in sampling to make sample match the population. Our main focus was to design a study in Caucasian population. In order to gain a representative sample, we used the below weighting factors according the acetylator gene: 60% for slow, 20 % for intermediate and 20% for fast acetylators.

Construction of elementary Fisher information matrix

In this thesis, we considered a combined error model for rifampicin and pyrazinamide:

$$y_i = f(\theta_i,\xi_i) + (1 + \varepsilon_{prop}) + \varepsilon_{add},$$

so the Σ n x n-diagonal matrix is restricted to the form :

$$\Sigma = \mathrm{diag}(\sigma^2_{add} + \sigma^2_{prop} \cdot f(\theta i, \xi i)^2),$$

And a proportional error model for isoniazid was assumed :

$$y_i = f(\theta_i,\xi_i) + (1 + \varepsilon_{prop}),$$

so Σ is restricted to the form:

$$\Sigma = \mathrm{diag}(\sigma^2_{prop} \cdot f(\theta i, \xi i)^2)$$

We applied the method of constructing the elementary Fisher Information Matrix as described by Retout et al.2002. We will describe the case of a combined error model for inter-individual random effects. The statistical model as we described in section 3.1.1 is:

$$y = f(\beta, b, \xi) + \varepsilon \cong f(\beta, 0, \xi) + \frac{\partial^T f(\beta,0,\xi)}{\partial b} b + \varepsilon$$

And then

$$E(y) \cong f(\beta, 0, \xi)$$

$$V(y) = V \cong \frac{\partial^T f(\beta,0,\xi)}{\partial b} \Omega \frac{\partial f(\beta,0,\xi)}{\partial b} + \Sigma \quad \text{(eq. 3)}$$

Where Σ are n x n-diagonal matrix and Ω is p x p-diagonal matrix.
The $\frac{\partial^T f(\beta,0,\xi)}{\partial b}$ is a n x p- matrix. Thus, the variance matrix has dimensions n x n.

In order to describe in detail the construction of variance of the vector of observation y, we write the (eq.3) in the below form.

V(y) =

$$\begin{bmatrix} \frac{\partial f(\beta,0,t_1)}{\partial b1} & \cdots & \frac{\partial f(\beta,0,t_1)}{\partial bp} \\ \vdots & \ddots & \vdots \\ \frac{\partial f(\beta,0,t_n)}{\partial b1} & \cdots & \frac{\partial f(\beta,0,t_n)}{\partial bp} \end{bmatrix} \begin{bmatrix} \omega_1^2 & \cdots & 0 \\ \vdots & \ddots & \vdots \\ 0 & \cdots & \omega_p^2 \end{bmatrix} \begin{bmatrix} \frac{\partial f(\beta,0,t_1)}{\partial b1} & \cdots & \frac{\partial f(\beta,0,t_n)}{\partial b1} \\ \vdots & \ddots & \vdots \\ \frac{\partial f(\beta,0,t_1)}{\partial bp} & \cdots & \frac{\partial f(\beta,0,t_n)}{\partial bp} \end{bmatrix} +$$

$$\begin{bmatrix} \sigma_{add}^2 + \sigma_{prop}^2 f(\beta,0,t_1)^2 & \cdots & 0 \\ \vdots & \ddots & \vdots \\ 0 & \cdots & \sigma_{add}^2 + \sigma_{prop}^2 f(\beta,0,t_n)^2 \end{bmatrix}$$

Where the $\frac{\partial f(\beta,0,t_k)}{\partial bj}$ is the finite difference for time k of the random parameter j and calculated by the formula

$$\frac{f(\beta,0,t_k) - f(\beta',0,t_k)}{h} \text{(eq.4)}$$

Where $\beta' = \beta \text{ x } \exp(b + h) = \beta \text{ x } \exp(0 + h)$, where $h = 10^{-5}$.

If we assume that $\lambda = (\omega_1^2, \omega_2^2, \dots, \omega_p^2, \sigma_{add}^2, \sigma_{prop}^2)^T$ the vector of variances, then $\Psi^T = (\beta^T \lambda^T)$. After some calculations, that there is no need to be described here (see Retout et al .2002), we ended up that the initial form of elementary Fisher information matrix (eq.1) is approximated by a block diagonal matrix :

$$M_F(\Psi, \xi) = \begin{bmatrix} A & 0 \\ 0 & B \end{bmatrix} = \begin{bmatrix} \frac{\partial^T f(\beta,0,\xi)}{\partial \beta} V^{-1} \frac{\partial f(\beta,0,\xi)}{\partial \beta} & 0 \\ 0 & \frac{F}{2} \end{bmatrix}$$

The expression $\frac{\partial f(\beta,0,\xi)}{\partial \beta}$ is also calculated by the formula in eq.4 and $\beta' = \beta + h$, where $h = 10^{-5}$.

Where F_{jk}= trace ($V^{-1} \frac{\partial V}{\partial \lambda j} V^{-1} \frac{\partial V}{\partial \lambda k}$). (eq.5)

The A is a p x p symmetric matrix for the fixed effects; B is a (p+2) x (p+2) symmetric matrix for the variances.
In order to develop the B matrix, as the expression of (eq.5) is complicated for straight calculation, we followed the simplifications that proposed by Retout et al. 2002.

i) First, for j and k in {1,….,p}

$$F_{jk}= \left(\frac{\partial f^{T}(\beta,0,\xi)}{\partial bj} V^{-1} \frac{\partial f(\beta,0,\xi)}{\partial \beta k} \right)^{2}$$

ii) Second, for k in {1,…..,p}

$$F_{(p+1)k}=F_{(p+2)k} = \left(\frac{\partial f^{T}(\beta,0,\xi)}{\partial bk} V^{-2} \frac{\partial f(\beta,0,\xi)}{\partial \beta k}\right)$$

iii) Third ,

$$F_{(p+1)(p+1)} =tr(V^{-2})$$

$$F_{(p+1)(p+2)}= F_{(p+2)(p+1)}=tr\ (V^{-2}y^{2})$$

$$F_{(p+2)(p+2)} = tr(V^{-2}y^{4})$$

Population FIM

In the present thesis, we assume that we will recruit 100 subjects. These subjects are separated in two different sampling cohorts. The population fisher information matrix for each one of the drugs are expressed by the below equation:

$$M_F(\Psi, \xi) =50xM_F(\Psi, \xi_1) + 50xM_F(\Psi, \xi_2)$$

Where ξ_1, ξ_2 are the designs for Cohort 1 and Cohort 2, respectively and50are the number of subjects in Cohort 1 and Cohort 2, respectively.

After the construction of the three Fisher information matrices (one for each drug), we calculated the determinants of each FIM. Since the FIM depends on the covariates, we calculated the average determinant of FIM with respect to the covariates. In order to do the averaging, we considered the 27 weight/age/genotype values of the distribution that we created (see section: I. Age and Weight, II. NAT2 enzyme) and we calculated

the determinant of FIM for each one of them. Finally, we calculated the mean of all determinants.

The particle swarm optimization algorithm is used for the optimization procedure. In this study, we want to optimize the sampling times for rifampicin, pyrazinamide and isoniazid, simultaneously. In order to optimize the design overall the 3 PK models concurrently, we averaged on the log scale the three determinants of each FIM, having first standardized them by the number of parameters in each model.

For this purpose, the below relationship has been used:

$$\Psi_{P-D} = \arg\max_{\Xi}\left(\sum_i^m \log\left(|M_F(\Psi,\Xi)|^{\alpha i/pi}\right)\right), \quad \text{(eq.6)}$$

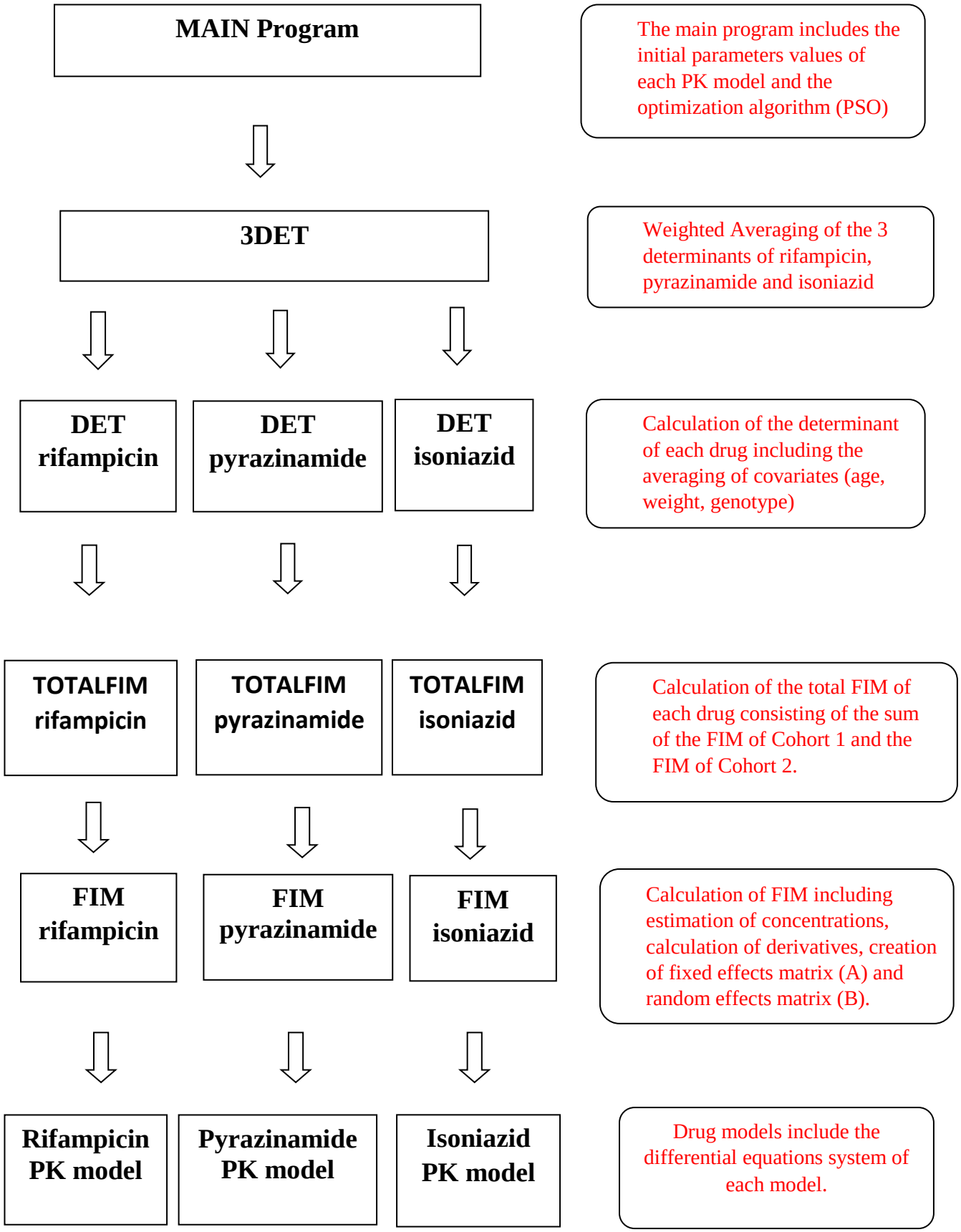
Where m are the number of models, a_i is the weighting in each model and p_i the parameters of the ith model and Ψ_i the parameter vector.

In the present thesis we use in the eq.6 so the parameter m is equal to 3, $a_{rifampicin} = a_{pyrazinamide} = a_{isoniazid} = 1$, as no particular weighting have been applied to the any of models and $p_{rifampicin} = 9$, $p_{ryrazinamide} = 11$, $p_{isoniazid} = 11$, are the number of parameters of rifampicin, pyrazinamide and isoniazid model, respectively.

The design optimization procedure was carried out using MATLAB2018a. All MATALB routines were original and written from scratch. For optimization the function `particleswarm` for PSO found in the Global Optimization Toolbox of MATLAB was used. The model evaluation procedure for the model of rifampicin, pyrazinamide and isoniazid was carried out via NONMEM software. Diagnostic plots (scatter plots and visual predictive check plots) were generated in Perl Speaks NONOMEM software (PsN) and Xpose (an R package).Bootstrap results were also generated by NONMEM software through PsN.

Software in MATLAB

A diagram of the software that we created in Matlab2018a is presented below:



4.3 RESULTS

Before the presentation of the common optimal sampling schedule for the three drugs concurrently, first we examined the sampling schedule for each drug, separately.

- Optimal design for rifampicin

The design region was set between 0.10 (the lower bound) and 6h (upper bound). As a sampling point under the 0.10h will give a low drug concentration (close to 0) and consequently this time point has no meaning for our design, we set the design region to begin 6minutes after drug administration. As the particle swarm algorithm does not require an elementary design, no initial times were provided to the algorithm.
First, we examined the case of an optimal sampling schedule with 6 sampling points for only one cohort. For rifampicin model, the optimal sampling schedule is:

$$Times_{optimal,rifampicin} = [\ 0.10h\ ,\ 0.51\ h\ ,0.51\ h,\ 1.40h,\ 2.10h\ ,6h]$$

While we asked for a 6 sampling point design, our optimization algorithm ended up to 5 different sampling points. It is obvious that we obtained a replicate of the second sampling point, as the algorithm provide us the more informative sampling times. Furthermore, we notice a characteristic of our algorithm; it tends to give the final sampling point close to upper bound that we defined. Second, we examined the case of a 5 sampling time design. The optimal sampling schedule that we obtained is:

$$Times_{optimal,rifampicin} = [\ 0.10h\ ,\ 0.57\ h,\ 1.42h,\ 2.17h\ ,\ 6h] \quad (eq.7)$$

The optimization procedure ended up to 5 different sampling points, similar to those obtained from the last case with no replicates this time.

As our aim is to design a study in two different cohorts of 50 children each, we examine the case of a sparser sampling schedule with 4 sampling times per subject. The optimal sampling schedule for the two cohorts for rifampicin drug is:

$$Times_{optimal,rifampicin,\ cohort1}=[0.10h,\ 0.93h,\ 2.0\ h,\ 6h]$$
$$Times_{optimal,rifampicin,\ cohort2}=[\ 0.56h,\ \ 1.44h,\ 2.17h\ ,\ 6h]$$

The optimal sampling points obtained are quite similar with those obtained by the optimization procedure for only one cohort (eq.7). If we decided to design a sparse clinical study for rifampicin with only 3 sampling points per patient the optimal sampling schedule would be:

$$Times_{optimal,rifampicin,\ cohort1}=[0.10h,\ 1.2h,\ 6h]$$
$$Times_{optimal,rifampicin,\ cohort2}=[0.56h,\ 1.41h,\ 1.99h]$$

As the algorithm found the optimal times for the two cohorts, we notice that the optimal sampling schedule does not differentiate to a great extend from the sampling schedule obtained by the 4 sampling design per subject.

- Optimal design for pyrazinamide

For pyrazinamide, we set up the particle swarm algorithm with the same properties as described in rifampicin's section. First, we examined the case of an optimal sampling schedule with 6 sampling points for only one cohort. The optimal sampling schedule is:

$$Times_{optimal,\ pyrazinamide}=[\ 0.10h\ ,\ 0.10\ h\ ,0.17\ h,\ 0.58h,\ 1.41\ h\ ,\ 6h]\quad (eq.8)$$

We noticed, that pyrazinamide's optimization gives 5 optimal times, while the first point (0.10h) is replicated. We understand that a more sparse design will be adequate for this drug. For this reason, we examined the case of a 5 sampling time schedule.
The optimal times that we obtained are:

$$Times_{optimal,\ pyrazinamide}=[\ 0.10h\ ,\ 0.16\ h,\ 0.57h,\ 1.41\ h\ ,\ 6h]$$

As we expected, the design is quiet similar to (eq.8), while the replicate of the first sampling point had been removed.
When we examined the case of 2 children cohorts with 4 samples per subject, the optimal times were:

$$Times_{optimal,\ pyrazinamide,cohort1} = [\ 0.10h,\ 0.17\ h,\ 0.54\ h,\ 1h]$$
$$Times_{optimal,\ pyrazinamide,cohort2} = [\ 0.10h,\ 0.10h\ ,\ 1.7h\ ,\ 6h]$$

The first sampling point (0.10h) is replicated 3 times for the cohort 2 sampling schedule, so we can continue examining an even sparser design with 3 samples per subject. The optimal sampling schedule for this design is:

$$Times_{optimal,\ pyrazinamide,cohort1} = [0.10h,\ 1.2h,\ 6h]$$
$$Times_{optimal,\ pyrazinamide,cohort2} = [0.56h,\ 1.41h,\ 1.99h]$$

- Optimal design for isoniazid

For isoniazid's model, we set up the particle swarm algorithm between time-interval [0.10h, 6h], for the same reason as described in rifampicin's section.As isoniazid's model has more fixed parameters than the other two models, it seemed reasonable to start exploration demanding more than 6 sampling points. We set up the algorithm to start exploration from 8 sampling points for one cohort. The optimal times that we obtained are:

$$Times_{optimal,\ isoniazid} = [\ 0.15h\ ,\ 0.50h,\ 1.19h\ ,1.19h\ ,2.29h\ ,\ 2.44\ h\ ,\ 4.33h\ ,\ 6h]$$

As some replicates are occurred, we continue the exploration with 7 sampling schedule and we obtained the below sampling schedule without any replicate.

$$Times_{optimal,\ isoniazid} = [\ 0.15\ h\ ,\ 0.50\ h,\ 1.14\ h\ ,\ 1.27h\ ,\ 2.42h\ ,4.35h\ ,\ 6h]$$

As we noticed the times are quiet similar with times obtained before, without the existence of any replicate this time.

Continuing to the same exploration pattern as we did with rifampicin and pyrazinamide model, a two cohort sampling schedule with 4 samples per subject is derived from the PSO algorithm and the below times were provided after optimization procedure;

$$Times_{optimal,\ isoniazid,\ cohort1} = [0.10h,\ 0.43\ h,\ 0.98\ h,\ 2.41h]$$
$$Times_{optimal,isoniazid,\ cohort2} = [0.16h,\ 2.05h\ ,\ 4.30\ h\ ,\ 6h]$$

And after the optimization procedure for 3 samples per patient, the optimal sampling schedule for the 2 cohorts are:

$$Times_{\ optimal,\ isoniazid,\ cohort1} = [0.16h,\ 1.58h,\ 6h]$$
$$Times_{\ optimal,\ isoniazid,\ cohort2} = [0.50h,\ 2.44h\ ,\ 4.34h]$$

- Optimal Design for Rifampicin-Isoniazid-Pyrazinamide

The aim of our work was to find a unique optimal time schedule for rifampicin, isoniazid and pyrazinamide so as this time schedule to be a good compromise for the three drugs, concurrently.

We set up the design region of the algorithm to allow a blood sampling schedule until 6 hours. Two different children cohorts of 50 children each were assumed with 4 samples per subject. After optimization, the optimal sampling times in Cohort 1 and Cohort 2 are

$$Times_{optimal,Cohort1} = [0.10h,0.13h,\ 0.55h\ ,\ 4.28h] \quad (eq.9)$$
$$Times_{optimal,Cohort2} = [0.57h\ ,\ 1.38h,\ 2.25\ h,\ \ 6h]$$

The optimal sampling times for the two cohorts have two sets of sampling points(0.10h and 0.13h) and (0.55 and 0.57h) which are too close. We notice the sampling time 0.10h is slightly differentiated from 0.13h only in the second decimal and the same issue is occurred in the set of 0.55h and 0.57h.This happens as the optimal sampling points for models without between-subject variability, should be

equal to the number of estimated fixed effects parameters. In models with between-subject variability, the number of optimal sampling points it depend on the underlying model structure but it is reasonable to assume that the number of sampling times to be equal or greater than the number of estimated fixed effects parameters.(Stromberg 2016). As the isoniazid's PK model include the most fixed effects of the 3 drugs (it consists of six estimated fixed effects; CL, V, MTT, ka , Q, Vper) ,the algorithm is trying to identify 6 optimal times. In optimal design evaluation, we also evaluated the accuracy of each model separately for the optimal times by using Visual Predictive Check Plots. VPCs were constructed based on 500 simulated replicates of the original dataset design. A unique bin was carried out with sampling points in eq.9 and also a bin for the six time points [0.115h, 0.56h, 1.38h, 2.25h, 4.28h ,6h]. The first and the second sampling point (0.115h, 0.56h) is an averaging of the two above mentioned sets. In FIGURE 13. - 15. , we presented the VPC plots for rifampicin, pyrazinamide and isoniazid model for the common design in eq.9.

The evaluation of the efficient criterion Φwas carried out in order to compare the optimal sampling design of each drug to the common sampling design obtain from the simultaneous optimization of the three drugs. Specifically, we set as Ξ_1 to be the optimal design of rifampicin for 2 cohorts with 4 samples per subject:

$$\Xi_1 = \begin{cases} 0.10\text{h}, 0.93\text{h}, 2.0\text{ h}, 6\text{h} \\ 0.56\text{h}, 1.44\text{h}, 2.17\text{h}, 6\text{h} \end{cases}$$

And as Ξ_2 the common design for the three drugs.

$$\Xi_2 = \begin{cases} 0.10\text{h}, 0.13\text{h}, 0.55\text{h}, 4.28\text{h} \\ 0.57\text{h}, 1.38\text{h}, 2.25\text{ h}, 6\text{h} \end{cases}$$

The criterion value was $\Phi = 1.12>1$, which correspond to a 12% gain in information from rifampicin's design compared to the common design.

The same criterion was applied for the optimal times of pyrazinamide and isoniazid comparing to the common design and resulted to an increase 12% gain in information for pyrazinamide's optimal times and 29% gain in information for the optimal times of isoniazid. We expected the additional increase of information in the model of

isoniazid as it is the model with the most fixed parameters. Nevertheless, the common design is a good compromise for the three drugs according to criterion Φ.

Optimal design evaluation

After the optimization procedure and having acquired the common sampling design for the 3 drugs concurrently (see eq.9), we would like to examine if this unique sampling design that we found is satisfactory. For this reason, we simulated a virtual clinical study, as if it is going to be implemented in reality. We analyzed this virtual study with NONMEM software and estimated the pharmacokinetic parameters. After that, we evaluated these estimations via diagnostic plots (scatter plots) and model evaluation techniques (VPC plots and Bootstrap method)

A dataset with 100 patients, according to the unique optimal design determined, was simulated. 50 subjects was set in Cohort 1 with sampling time points at 0.10h,0.13h, 0.55h and 4.28h and the other 50 subjects in Cohort 2 with sampling time points 0.57h , 1.38h, 2.25 h and 6h. The dosage that administered to each subject was decided according to the respective weight band (see paragraph I. Age and Weight)

- **Goodness of Fit plots**

In FIGURE 10.-12., scatter plots of individual predicted concentrations versus the observed concentrations for rifampicin, pyrazinamide and isoniazid models are presented for the common design of the 3 drugs. The coefficient of determination R^2 is greater than 97.5 % for all models, so we have a first indication of a goodness of fit.

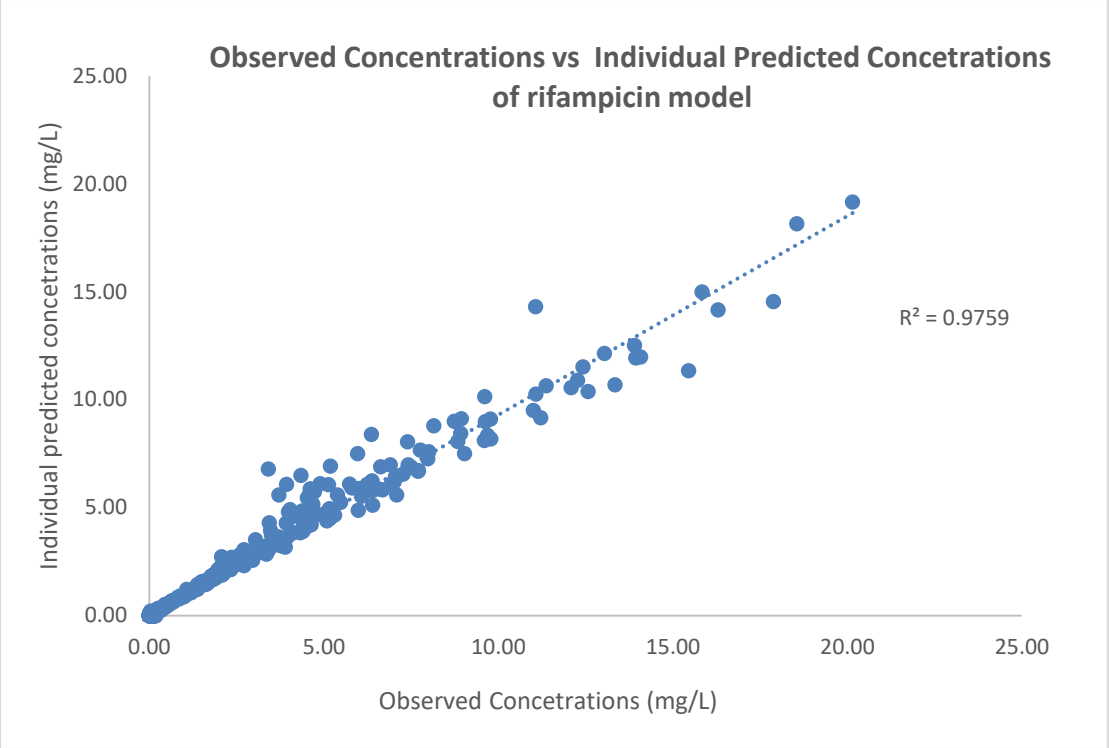


FIGURE 10. SCATTER PLOT OF INDIVIDUAL PREDICTED CONCENTRATIONS VERSUS THE OBSERVED CONCENTRATIONS FOR RIFAMPICIN MODEL

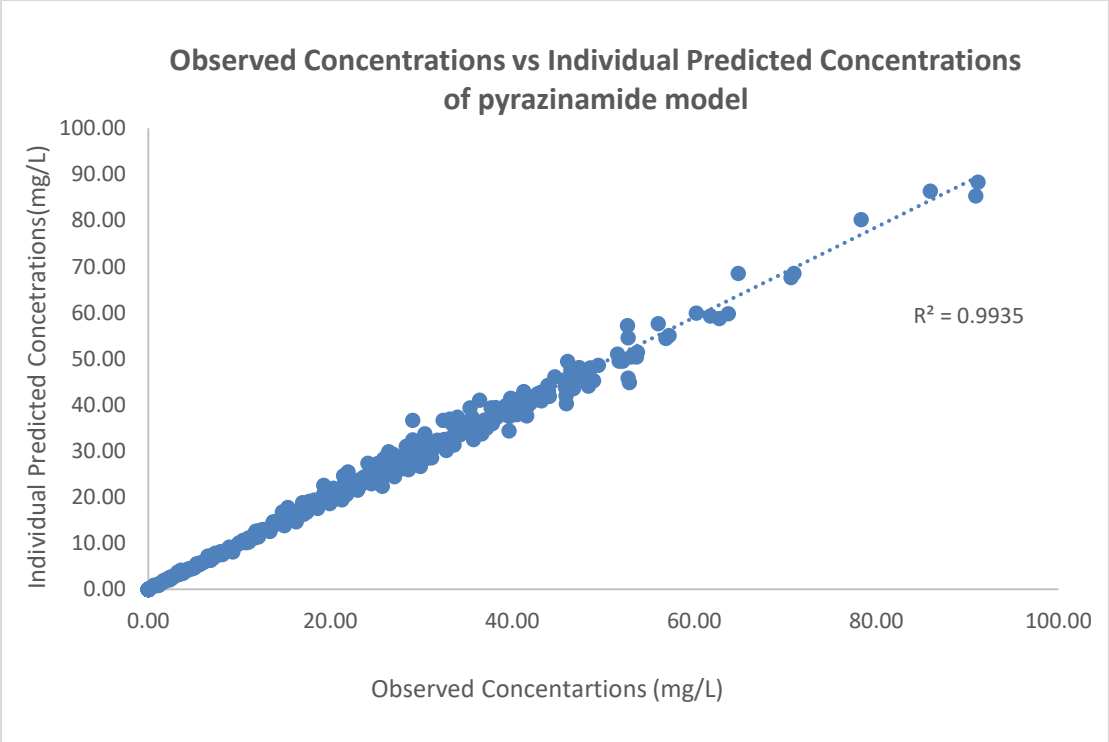


FIGURE 11. SCATTER PLOT OF INDIVIDUAL PREDICTED CONCENTRATIONS VERSUS THE OBSERVED CONCENTRATIONS FOR PYRAZINAMIDE MODEL

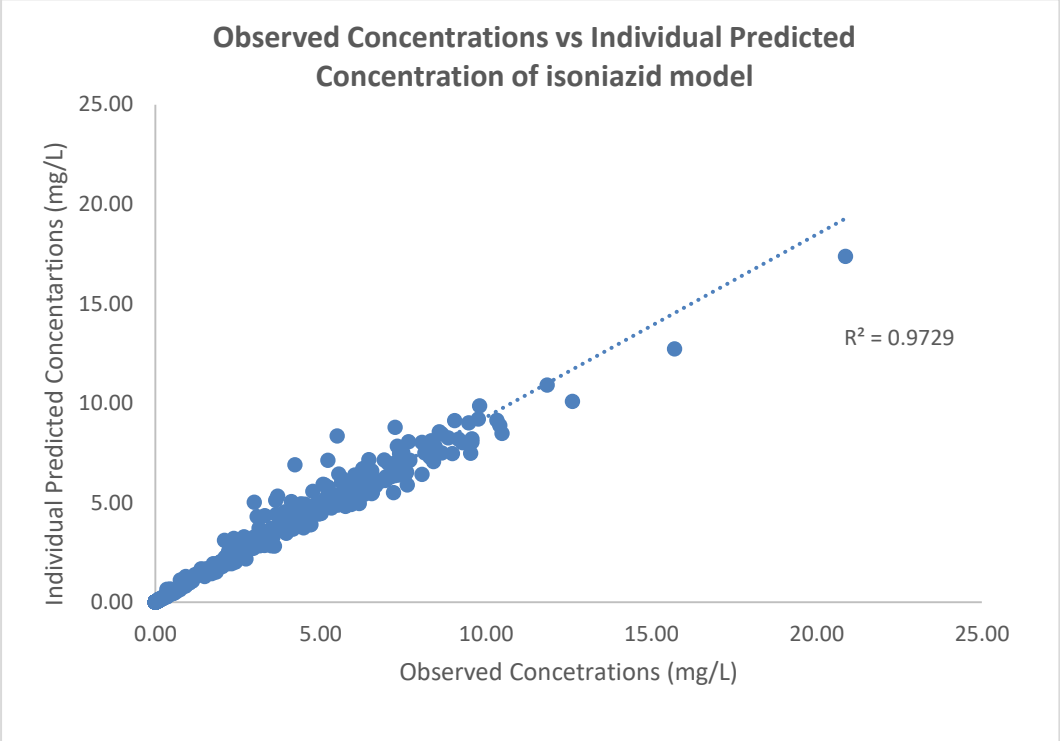


FIGURE 12. SCATTER PLOT OF INDIVIDUAL PREDICTED CONCENTRATIONS VERSUS THE OBSERVED CONCENTRATIONS FOR ISONIAZID MODEL

- **Visual predictive check plots**

In order to evaluate our PK models, the corresponding visual predictive check plots (VPC) for each drug is presented below.

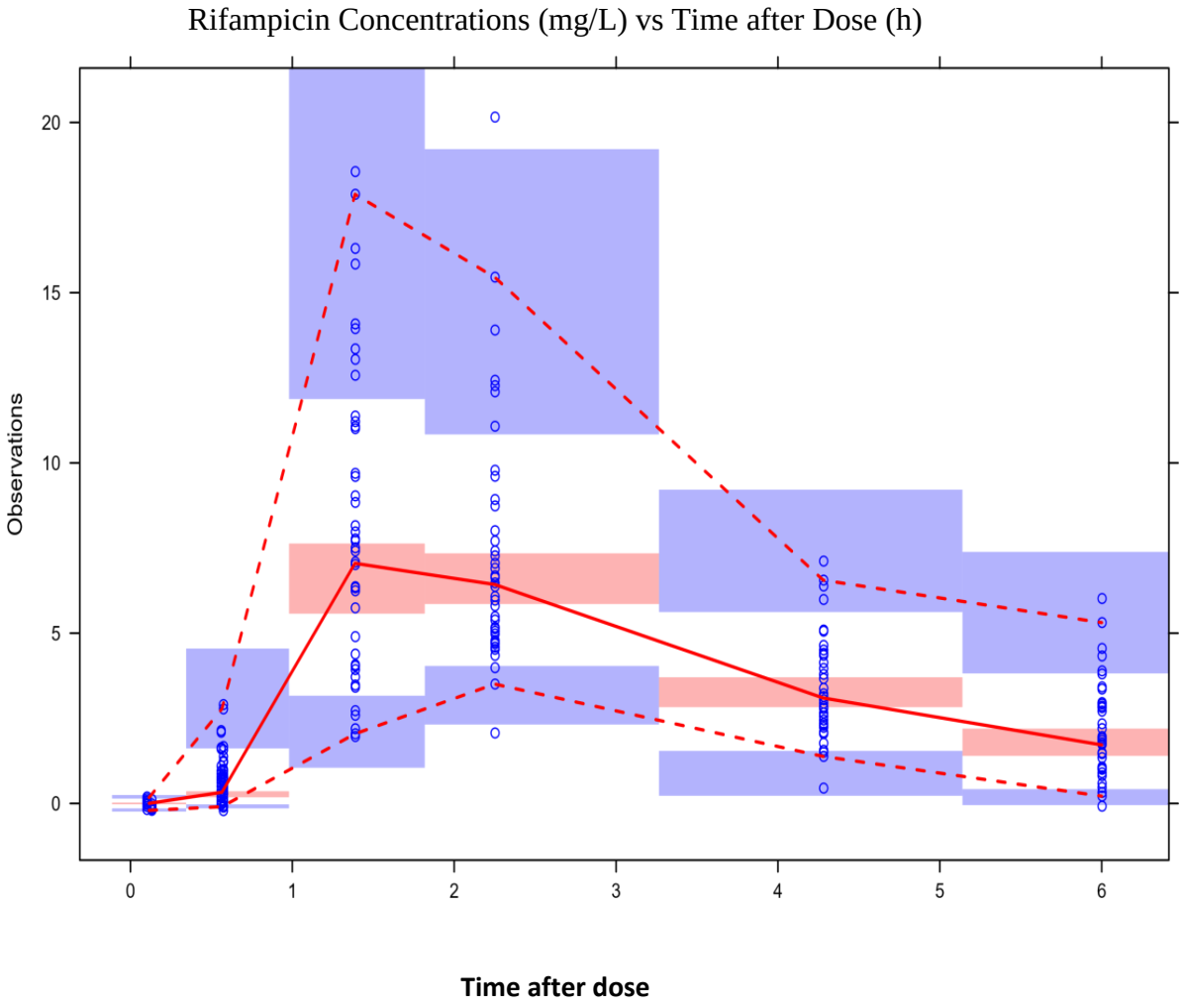


FIGURE 13. VPC FOR THE RIFAMPICIN MODEL.

The blue dots (o) presented are the observations (rifampicin concentrations).

The lower, middle and upper lines are the 2.5th percentile, median (50th percentile) and 97.5th percentile of the observed data. Median and percentiles are plotted at the mean time since dose of the data observed within each time since dose interval. The blue shaded areas are the 95% CI for the 2.5th percentile and 97.5th percentile and the pink shaded area is the 95% CI for the median of simulated data.

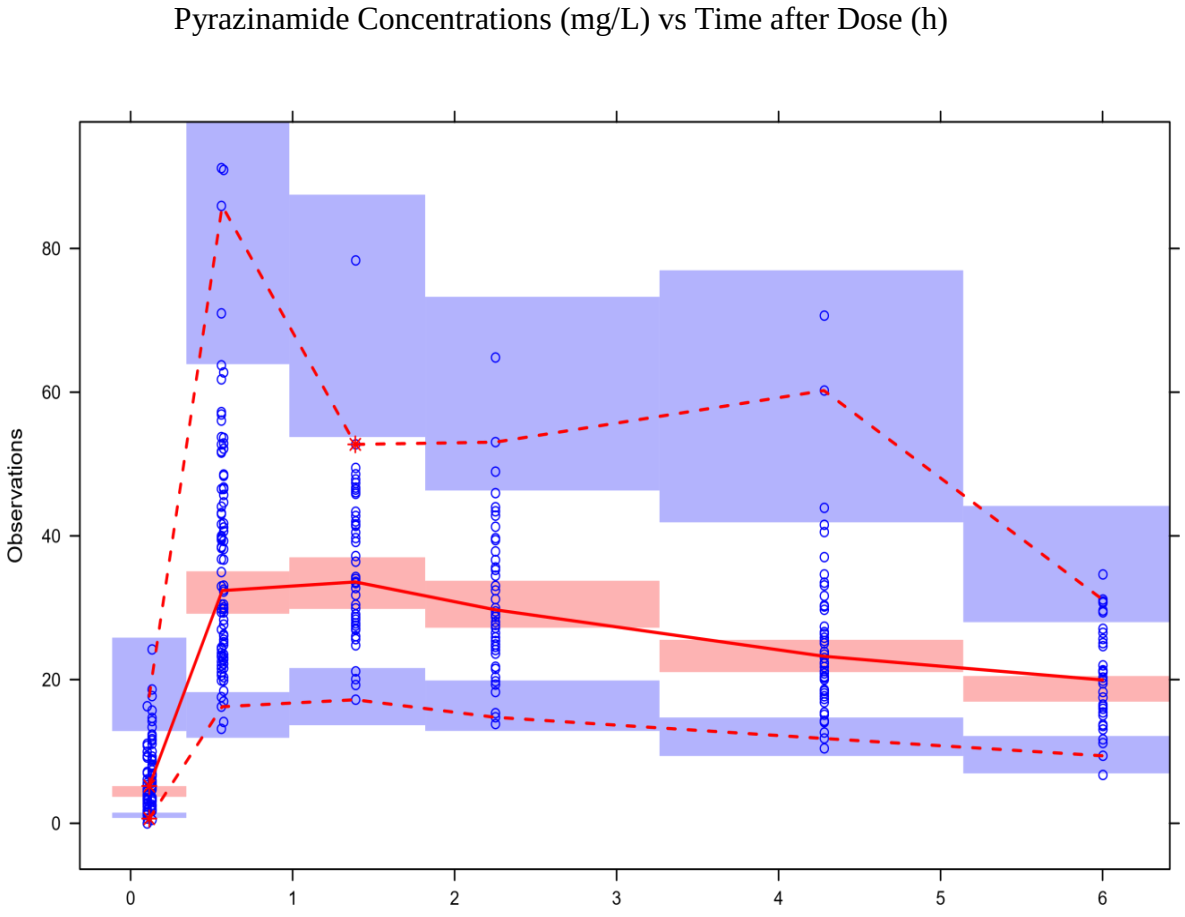


FIGURE 14.VPC FOR THE PYRAZINAMIDE MODEL.

The blue dots (o) are presented the observations (pyrazinamide concentrations).

The lower, middle and upper lines are the 2.5th percentile, median (50th percentile) and 97.5th percentile of the observed data. Median and percentiles are plotted at the mean time since dose of the data observed within each time since dose interval. The blue shaded areas are the 95% CI for the 2.5th percentile and 97.5th percentile and the pink shaded area is the 95% CI for the median of simulated data.

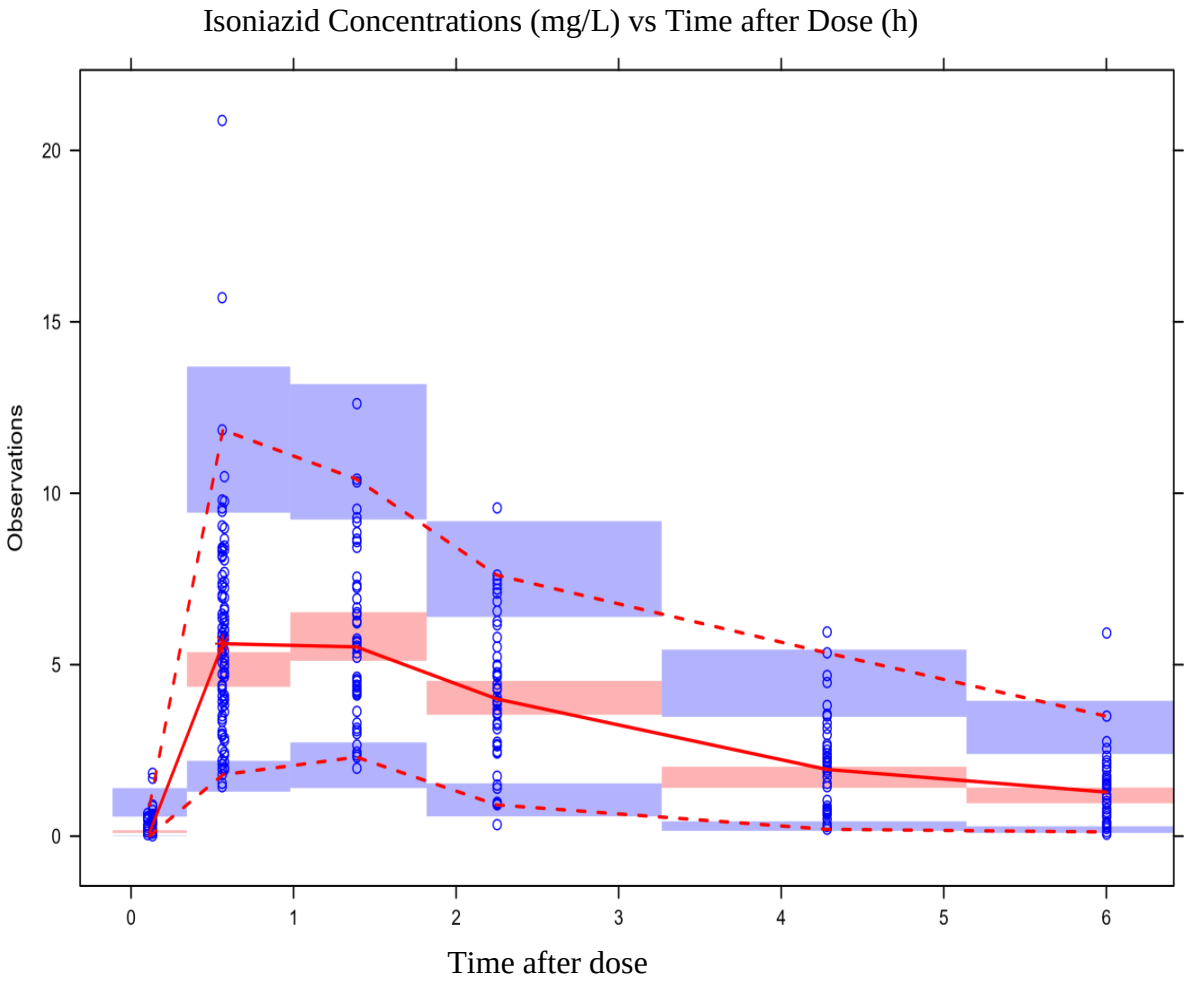


FIGURE 15. VPC FOR THE ISONIAZID MODEL.

The blue dots (o) are presented the observations (isoniazid concentrations).

The lower, middle and upper lines are the 2.5th percentile, median (50th percentile) and 97.5th percentile of the observed data. Median and percentiles are plotted at the mean time since dose of the data observed within each time since dose interval. The blue shaded areas are the 95% CI for the 2.5th percentile and 97.5th percentile and the pink shaded area is the 95% CI for the median of simulated data.

For all pharmacokinetic models, the VPC results are supportive of the respective model. The median of the simulated prediction tracks are very well with the median of the observed data, across the entire dosing interval, and the 95% CI for the 2.5th percentile and 97.5th percentile of simulated data are also fairly consistent with corresponding percentiles based on data. In only the third time bin of pyrazinamide,

the 97.5th percentile of the data falls slightly outside the 95% CI around the upper prediction interval.

- **Bootstrap**

500 bootstrap datasets were generated by replacement, from the 100 simulated datasets for the three drugs. Bootstrap results for the three PK models and presented below.

TABLE 6. INITIAL PARAMETERS VALUES, NONMEM PARAMETER ESTIMATES AND BOOTSTRAP RESULTS FOR RIFAMPICIN

Parameters (units)	Initial Parameter values	NONMEM Parameter Estimates (RSE(%))	Bootstrap		
			Median	95 % CI	RSE(%)
β_{CL}(L/h)	8.15	9.10(3%)	8.94	7.88-11.93	9%
β_{V}(L)	16.2	18.20(6%)	18.16	16.08-21.07	7%
β_{MTT}(h)	1.04	1.03(3%)	1.02	0.96 -1.07	3%
β_{n}	8.04	7.46(6%)	7.49	6.54-8.62	7%
β_{tm50} (wks)	58.2	57.10(7%)	57.78	46.67-83.57	15%
β_{HILL}	2.21	1.77(6%)	1.93	1.02-3.52	32%
ω^2_{CL}	0.09	0.09(22%)	0.08	0.04-0.11	23%
ω^2_{V}	0.16	0.16(25%)	0.16	0.09-0.24	22%
ω^2_{MTT}	0.04	0.035(18%)	0.03	0.02-0.04	18%
σ^2_{add}(mg/L)^2	0.01	0.01(11%)	0.01	0.008-0.012	10%
σ^2_{prop}	0.04	0.038(18%)	0.03	0.02 - 0.06	23%

TABLE 7. INITIAL PARAMETERS VALUES, NONMEM PARAMETER ESTIMATES AND BOOTSTRAP RESULTS FOR PYRAZINAMIDE

Parameters (units)	Initial Parameter Values	NONMEM Parameter Estimates(RSE(%))	Bootstrap		
			Median	95% CI	RSE(%)
β_{CL}(L/h)	1.08	1.21(4%)	1.20	1.12-1.30	3%
β_{V}(L)	9.64	9.40(4%)	9.36	8.79-10.1	3%
$\beta_{ka}(h)^{-1}$	4.48	4.08(7%)	4.08	3.64-4.74	6%
β_{MTT}(h)	0.10	0.09(8%)	0.09	0.08-0.10	6%
β_{n}	3.94	4.73(26%)	4.69	3.14-6.71	19%
ω^2_{CL}	0.09	0.082(17%)	0.08	0.05-0.10	17%
ω^2_{V}	0.09	0.11 (14%)	0.10	0.07-0.13	14%
ω^2_{ka}	0.09	0.13(28%)	0.12	0.05-0.20	28%
ω^2_{MTT}	0.09	0.082(26%)	0.07	0.04-0.13	27%
$\sigma^2_{add}(mg/L)^2$	0.01	0.02(50%)	0.02	0.006-0.05	47%
σ^2_{prop}	0.01	0.007(14%)	0.007	0.005-0.009	13%

TABLE 8.INITIAL PARAMETERS VALUES, NONMEM PARAMETER ESTIMATES AND BOOTSTRAP RESULTS FOR ISONIAZID

Parameters (units)	Initial Parameter Values	NONMEM Parameter Estimates(RSE(%))	Bootstrap		
			Median	95% CI	RSE(%)
β_{CL}(L/h)	4.44	4.79(6%)	4.79	4.15-5.62	8%
β_{V}(L)	11.0	9.87(12%)	9.82	6.57-11.9	13%
β_{ka} $(h)^{-1}$	2.47	2.10(15%)	2.12	1.38-2.81	17%
β_{MTT}(h)	0.179	0.18(4%)	0.18	0.16-0.20	4%
β_{Q}	2.00	2.60(32%)	2.52	1.59-5.00	34%
β_{Vper}	5.03	4.82(15%)	5.27	4.05-9.37	36%
$\beta_{Fim/fast}$	0.772	0.799(6%)	0.79	0.70-0.89	6%
β_{TM50}(wks)	49.0	48.8(7%)	49.5	42.9-62.3	10%
β_{Hill}	2.19	2.49(30%)	2.47	1.39-5.28	51%
ω^2_{CL}	0.09	0.08(16%)	0.08	0.05-0.11	19%
ω^2_{V}	0.09	0.16(29%)	0.15	0.004-0.28	34%
ω^2_{ka}	0.25	0.08(75%)	0.09	0.05-0.26	64%
ω^2_{MTT}	0.09	0.08(20%)	0.09	0.05-0.12	18%
σ^2_{prop}	0.04	0.03(13%)	0.03	0.02-0.04	11%

According to the table results for the three PK models, we have to discuss and comment two different points.

The first of them is the precision and accuracy, as regards the difference between the initial parameter values obtained from literature (Initial Parameter Values) and the final parameter estimates obtained by NONMEM. For rifampicin model, for all fixed effects the difference between the initial parameters and final estimates does not exceed the 20%, for pyrazinamide model the 16% and for isoniazid model the 23%. The variance of random effects were also close to NONMEM parameter estimates for the three PK models, except for absorption rate constant in isoniazid model which is underestimated. The residual variability was under the 50% difference for all three models. The relative standard errors (RSE) for rifampicin model obtained by the NONMEM are precise. For the fixed effects, RSE are under 10% and for the random effects are under 30%.For the model of pyrazinamide, all RSE for fixed effects were under 26% and for the random effects under 30%. For isoniazid model, RSE are also precise even though for inter-compartmental clearance parameter and Hill factor parameter the RSE were at the limit of 30% and the RSE of absorption rate constant parameter is also increased (75%). This can be explained as the isoniazid model, were evaluated consisted of 11 parameters (including 9 fixed effects parameters)and can be considered as over-parameterized. Nevertheless, the RSE shown precision for all 3 models, as the population mean parameters were within acceptance levels (defined as <30% for mean population parameters and <50 % for variance parameters values), except for k_a in isoniazid model.

The second point that has to be commented is the results obtained by bootstrap procedures. Bootstrap is a very robust method, gets reliable estimates for RSE and CI than the NONMEM, considering as “the gold standard” in design evaluation. Being in line with NONMEM results, RSE shown precision for the most pharmacokinetic parameters of the rifampicin, pyrazinamide and isoniazid model. More particularly, for rifampicin model, bootstrap results present small RSE (under 15%), apart from Hill factor that is 32%. For pyrazinamide model, all parameter estimates are unbiased. In isoniazid model, Hill Factor is increased to 51% while all the other population parameters are under from 36%. Bootstrap results showed that the variance of rate absorption constant is also increased (64%). A similar increase has been also noticed in NONMEM results. For both in model of isoniazid and rifampicin, we have to stand

and comment the results of the Hill factor parameter. The reason that this particular population parameter tends to have a bigger variability than the other fixed parameters lies to the fact that Hill factor is connected with the organ maturation process (maturation for oral clearance and MTT). As maturation process for children is reaching a plateau after the 6 months after birth, Hill factor stops playing a role. Nonetheless, in our study we have included children aged 1 month after birth and older, so Hill factor parameter had to be added in our model.

4.4 DISCUSSION

In the present work, D-optimality was the criterion used for the sampling design optimization procedure and the particle swarm optimization was the algorithm used for optimization. Particle swarm optimization algorithm has not been implemented before for the optimization procedure in the field of pharmacokinetics. This is the first time that this algorithm is used for optimization in pharmacokinetic studies.

However, particle swarm was not the only algorithm that we used for optimization in the present thesis. A number of optimization algorithms are known from literature for designing optimal population pharmacokinetic studies. A common algorithm used is the Fedorov-Wynn algorithm which uses only a finite number of sampling times, which have been determined from the design region therefore leading to the selection of a local optimal design. Simplex algorithm, adaptive random search and simulating annealing (SA) are some algorithms that have also used.(Ogungbenro et al. 2005).
Simulating annealing algorithm was the one that we applied in our work before the selection of particle swarm algorithm. Inspired by thermodynamics, SA is a stochastic derivative free minimization algorithm that search over the entire surface of the determinant and allows both upward and downward steps, although certain intrinsic variables change the range of uphill step. Uphill progress is controlled by Metropolis step. Although, it has been proposed as a superior and robust algorithm comparing to other algorithms such as simplex (Duffull et at. 2002), in our work some weaknesses appeared. This weakness lies to the fact that SA needs an elementary design to be provided. If the initial design is different in optimization procedure, the algorithm fails to find exactly the same optimal sampling times (especially the optimal sampling times close to upper bound).As opposed to SA, in the particle swarm algorithm there

is no need of initial design and in every optimization run, the algorithm performed better, ending up to the same optimal sampling schedule every time.

It is worth mentioning that, even when we do not averaging out the covariates (age,etc.) and gave them a fixed value, the particle swarm algorithm ended up approximately to the same optimal sampling points (a difference on the first or second decimal may be appeared).

Generally, the robustness of the optimal design and the model specification is an important problem in the drug research. The optimization done in this study is model dependent. Although the three models used in this study are based on *a priori* information given by previous study which uses these PK models(Zvada et al. 2014), it would be interesting to test the robustness of the design on other models e.g. for different random effects models.

Moreover, several modifications can also be done in the software that we created from scratch in MATLAB, according to user's needs. For example, we created an even sparser common design schedule for the 3 drugs simultaneously with optimal times for each cohort the below:

$$Times_{optimal,Cohort1} = [\ 0.10h,\ 3.73h,\ 6h]$$

$$Times_{optimal,Cohort2} = [\ 0.51h,\ 1.13h,\ 1.80h]$$

An even sparser design (e.g. 2 blood samples per subject) or the addition of one or more cohorts of subjects can also be applied to the software, with some modifications in the MATLAB routines. The number of subjects in each cohort can also be optimized, but caution should be taken in order to keep the design robust. Finally, the software that we created can also be used for the optimization design procedure of other drugs, apart from rifampicin, pyrazinamide and isoniazid.

4.5 CONCLUSIONS

The final conclusions of the present work are; a common optimal design for a pediatric PK study can be obtained using D-optimal design technique for 3 drugs simultaneously. The sampling time schedule in which we finally concluded is: 0.10h, 0.13h, 0.55h and 4.28h for Cohort 1 and 0.57 h, 1.38h, 2.25h and 6h for Cohort 2. This unique time schedule is a good compromise for rifampicin, pyrazinamide and isoniazid, concurrently and the evaluation of the 3 pharmacokinetic models shown accurate results, as regards the parameter estimates.

Concluding, the general purpose of this work is that; via modelling and simulation techniques virtual clinical studies can be designed. Optimizing drug doses, blood sampling times, number of subjects etc. pharmaceutical industries can save money and time from designing inappropriate clinical studies.

According to our work, if this clinical trial that we designed is implemented in Europe or US, the FDC product for the treatment of tuberculosis that is currently available only in deprived countries, will take Marketing Authorization Approval and will be available also in countries of Europe and US.

ABSTRACT

The objective of this dissertation was to design a sparse sampling pediatric pharmacokinetic study for a fixed dose combination product of isoniazid (H), rifampicin (R) and pyrazinamide (Z) for the treatment of tuberculosis. A single dose of FDC tablet was supposed to be given into two cohorts of fifty (50) children each. Non-linear mixed effects models were used to describe the structure of each drug model. We determined a unique optimal sampling schedule for the three drugs, such that the parameters of the PK models of each drug are estimated with high precision. We applied a method based on an expression for the Fisher Information Matrix (FIM) for non-linear mixed effects to improve the sampling design so as to obtain efficient parameter estimates. The approach is based on Rao-Cramer inequality which states that the inverse of FIM is the lower bound of the variance-covariance matrix of any unbiased estimators of the parameters. The criterion used for the optimization is D-optimality; a design is considered D-optimal if it maximizes the determinant of the Fisher information matrix. The particle swarm optimization (PSO) algorithm was applied for the optimization procedure while all implementation was conducted in MATLAB. The final design was evaluated by simulations and estimation with NONMEM. Bootstrap, Visual Predictive Check (VPC) plots and Goodness of fit plots were generated.

A pharmacokinetic study with 4 blood samples per subject was eventually designed. The optimal blood sampling times for the first cohort is0.10h, 0.13h, 0.55h and 4.28h and optimal blood sampling times for Cohort 2 is 0.57 h, 1.38h, 2.25h and 6h.The evaluation of the 3 pharmacokinetic models showed accurate results, as regards the parameter estimates.

Finally, if the clinical trial that we designed is implemented, it could be used for taking Marketing Authorization Approval of first-line paediatric fixed dose combination product for the treatment of tuberculosis in Europe and USA, which is currently unavailable.

ΠΕΡΙΛΗΨΗ

Ο σκοπός αυτής της εργασίας είναι να σχεδιαστεί μια παιδιατρική φαρμακοκινητική μελέτη αραιής δειγματοληψίας ενός φαρμακευτικού σκευάσματος σταθερού συνδυασμού δόσης, ισονιαζίδης, ριφαμπικίνης και πυραζιναμίδης για την θεραπεία της φυματίωσης. Θεωρείται ότι θα δοθεί μια μοναδική χορήγηση του φαρμακευτικού σκευάσματος σε δυο κοορτές των πενήντα(50) παιδιών η καθεμία. Μη γραμμικά μοντέλα μικτών επιδράσεων χρησιμοποιήθηκαν προκείμενου να περιγράψουν την δομή καθενός από τα φαρμακευτικά μοντέλα. Καθορίσαμε ένα μοναδικό δειγματοληπτικό σχήμα για τα τρία φάρμακα, έτσι ώστε οι παράμετροι των φαρμακοκινητικών μοντέλων να εκτιμηθούν με υψηλή ακρίβεια. Εφαρμόστηκε μια μέθοδος βασισμένη στον Πίνακα Πληροφορίας του Fisher (Fisher Information Matrix) για μη γραμμικά μοντέλα μικτών επιδράσεων προκειμένου να βελτιστοποιηθούν οι χρόνοι δειγματοληψίας αποκτώντας αποτελεσματικές εκτιμήσεις των παραμέτρων. Η προσέγγιση αυτή βασίζεται στην ανισότητα του Rao-Cramer κατά την οποία ο αντίστροφος του Πίνακα Πληροφορίας του Fisher είναι το κάτω φράγμα του πίνακα διασποράς- συν διασποράς κάθε αμερόληπτού εκτιμητή των παραμέτρων. Το κριτήριο που χρησιμοποιήθηκε για την βελτιστοποίηση είναι το D-βέλτιστο. Ένας σχεδιασμός θεωρείται D-βέλτιστος εάν μεγιστοποιεί την ορίζουσα του Πίνακα Πληροφοριών του Fisher. Ο αλγόριθμος particle swarm optimization (PSO) εφαρμόστηκε για την διαδικασία της βελτιστοποίησης καθώς και όλη η εφαρμογή διεξήχθηκε στο προγραμματιστικό πακέτο MATLAB. Ο τελικός σχεδιασμός αξιολογήθηκε μέσω προσομοιώσεων και εκτιμήσεων στο πακέτο NONMEM. Bootstrap, Visual predictive check plots και γραφήματα καλής προσαρμογής δημιουργήθηκαν.

Τελικά σχεδιάστηκε, μια φαρμακοκινητική μελέτη με 4 δείγματα αίματος ανά ασθενή. Οι βέλτιστοι χρόνοι δειγματοληψίας για την πρώτη κοορτή είναι στις 0.10 , 0.13 ,0.55 και 4.28 ώρες και οι βέλτιστοι χρόνοι δειγματοληψίας για την δεύτερη κοορτή είναι στις 0.57 , 1.38, 2.25 and 6 ώρες.

Η αξιολόγηση των τριών φαρμακοκινητικών μοντέλων έδειξε ακριβή αποτελέσματα, όσο αναφορά τις εκτιμήσεις των παραμέτρων.

Κλείνοντας, εάν αυτή η κλινική μελέτη πραγματοποιηθεί, τότε το φαρμακευτικό αυτό σκεύασμα σταθερού συνδυασμού δόσης για την θεραπεία της φυματίωσης, θα μπορεί

να πάρει έγκριση για άδεια κυκλοφορίας στην Ευρώπη και την Αμερική, το οποίο μέχρι τώρα δεν είναι διαθέσιμο.

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APPENDIX

Matlab Code

```
% Main_Program
clear;
wt=[4.5,5.6,6.4,7,7.5,7.9,8.3,9.2,9.9,10.5,11.1,11.8,12.4,12.9,13.5,1
4,14.5,15,15.5,16,17,18,19,20.1,21.3,22.5,23.7];
pma=[40,44,48,52,56,60,64,76,88,100,112,124,136,148,160,172,184,196,2
08,220,244,280,292,316,340,364,388];
acetfactor=[
1,2.013,2.013,1,1,2.54,2.54,1,2.54,1,1,2.013,2.54,2.0130,1,1,1,1,1,2.
013,1,1,1,1,2.54,1,1];
ffactor=[1,0.772,0.772,1,1,0.772,0.772,1,0.772,1,1,0.772,0.772,0.772,
1,1,1,1,1,0.7720,1,1,1,1,0.772,1,1];
fori=1:length(wt)
ifwt(i)<=7.9
tablet(i)=1 ;
elseifwt(i)>=8  &&wt(i)<=11.9
tablet(i)=2;
elseifwt(i)>=12    &&wt(i)<=15.9
tablet(i)=3;
else
tablet(i)=4;
end
end
riftheta(1)=8.15 ;
riftheta(2)=16.2;
riftheta(3)=1.04;
riftheta(4)=8.04;
rifsigma(1)=0.2;
rifsigma(2)=0.1;
rifwmega(1)=0.3;
rifwmega(2)=0.4;
rifwmega(3)=0.2;
pyraztheta(1)=1.08 ;
pyraztheta(2)=9.64;
pyraztheta(3)=4.48;
pyraztheta(4)=0.1;
pyraztheta(5)=3.94;
pyrazwmega(1)=0.3;
pyrazwmega(2)=0.3;
pyrazwmega(3)=0.3;
pyrazwmega(4)=0.3;
pyrazsigma(1)=0.1;
pyrazsigma(2)=0.1;
isontheta(1)=4.4;      %cl
isontheta(2)=11;       %v
isontheta(3)=2.47;     %ka
isontheta(4)=0.179;    %mtt
isontheta(5)=2;        %Q
isontheta(6)=5.03;     %V periph
isonsigma(1)=0.2;
isonwmega(1)=0.3;  %wcl
isonwmega(2)=0.3;   %wv
isonwmega(3)=0.5;  %wka
isonwmega(4)=0.3;  %wmtt
```

```
options = optimoptions('particleswarm','UseParallel',1)
times=particleswarm(@(times)finalorizD2Coh3drugs_with_wt(times,rifthe
ta,pyraztheta,isontheta,rifwmega,pyrazwmega,isonwmega,rifsigma,pyrazs
igma,isonsigma,acetfactor,ffactor,tablet,wt,pma),8,[0.1 0.1 0.1 0.1
0.1 0.1 0.1 0.1],[ 6 6 6 6 6 6 6 6],options)
```

% 3DET

```
function
finaloriz2Coh3drugs=finalorizD2Coh3drugs_with_wt(times,riftheta,pyraz
theta,isontheta,rifwmega,pyrazwmega,isonwmega,rifsigma,pyrazsigma,iso
nsigma,acetfactor,ffactor,tablet,wt,pma)
finalriforiz=finalDrif2cohort(times,riftheta,rifwmega,rifsigma,tablet
,wt,pma);
finalpyrazoriz=finalDpyraz2cohort(times,pyraztheta,pyrazwmega,pyrazsi
gma,tablet,wt);
finalisonoriz=finalDison2cohort(times,isontheta,isonwmega,isonsigma,a
cetfactor,ffactor,tablet,wt,pma);
finaloriz2Coh3drugs=(finalriforiz/9 +finalpyrazoriz/11 +
finalisonoriz/11);
disp(times);
disp(finaloriz2Coh3drugs)
disp(finalriforiz)
disp(finalpyrazoriz)
disp(finalisonoriz)
end
```

% DET rifampicin

```
function
finalriforiz=finalDrif2cohort(times,riftheta,rifwmega,rifsigma,tablet
,wt, pma)
oriz=zeros(length(wt));
parfori=1:length(wt)
oriz(i)=finalriftwoCohortDfim(times,riftheta,rifwmega,rifsigma,tablet
(i),wt(i),pma(i))
end
finalriforiz=-real(mean(log(-oriz)))
disp(times);
end
```

%TOTALFIM rifampicin

```
function
oriz=finalriftwoCohortDfim(times,riftheta,rifwmega,rifsigma,tablet,wt
,pma)
times1=times(1:4);%  or times1=times(1:3);
times2=times(5:8);%  or times2=times(4:6);
p=0.5;
totalFim1=riftotalFim(times1,riftheta,rifwmega,rifsigma,tablet,wt,
pma);
```

```
totalFim2=riftotalFim(times2,riftheta,rifwmega,rifsigma,tablet,wt,
pma);
totalfim=p*totalFim1 +(1-p)*totalFim2 ;
oriz=-det(totalfim);
end
```

% FIM rifampicin

```
functiontotalFim=
riftotalFim(times,riftheta,rifwmega,rifsigma,tablet,wt, pma)
A=rifA(times,riftheta,rifwmega,rifsigma,tablet,wt, pma);
B=rifB(times,riftheta,rifwmega,rifsigma,tablet,wt, pma);
totalFim=(1/2)*blkdiag(A,B);
end


function A=rifA(times,riftheta,rifwmega,rifsigma,tablet,wt, pma)
dydz = deriv(times,riftheta,tablet,wt, pma);
Var=rifVar(times,riftheta,rifwmega,rifsigma,tablet,wt, pma);
A=2*dydz'*(inv(Var))*dydz ;
End



function B=rifB(times,riftheta,rifwmega,rifsigma,tablet,wt, pma)
dydb=derivb(times,riftheta,tablet,wt, pma);
y = conc(times,riftheta,tablet,wt, pma);
Var=rifVar(times,riftheta,rifwmega,rifsigma,tablet,wt, pma);
Fsasa=trace(Var^-2);
Fsasp=trace((Var^-2)*diag(y.^2));
Fspsa=trace((Var^-1)*diag(y.^2)*(Var^-1));
Fspsp=trace((Var^-2)*diag(y.^4));
Fp1k=rifFp1k(times,riftheta,rifwmega,rifsigma,tablet,wt, pma);
Fjk=(dydb'*(Var^-1)*dydb)^2;
B=[Fjk,Fp1k',Fp1k';Fp1k,Fsasa,Fsasp;Fp1k,Fspsa,Fspsp];
End



function Fp1k=rifFp1k(times,riftheta,rifwmega,rifsigma,tablet,wt,
pma)
dydb=derivb(times,riftheta,tablet,wt, pma);
Var=rifVar(times,riftheta,rifwmega,rifsigma,tablet,wt, pma);
Fp1k=zeros(1,length(riftheta)-1);
for k=1:length(riftheta)-1
    Fp1k(:,k)=dydb(:,k)'*(Var^(-2))*dydb(:,k);
End


functionVar=rifVar(times,riftheta,rifwmega,rifsigma,tablet,wt, pma)
w=WMEGA(rifwmega);
s=S(times,riftheta,rifsigma,tablet,wt, pma);
dydb = derivb(times,riftheta,tablet,wt, pma);
Var=dydb*w*dydb' + s;
end
```

```
function s=S(times,riftheta,rifsigma,tablet,wt, pma)
s=diag(conc(times,riftheta,tablet,wt, pma)*rifsigma(1).^2 +
rifsigma(2).^2);
end


function w=WMEGA(rifwmega)
w=diag(rifwmega.^2);
end


functiondydb=derivb(times,riftheta,tablet,wt, pma)
dydb=zeros(length(times),length(riftheta)-1);
fori=1:length(riftheta)-1
h=0.00001;
theta2=riftheta;
theta2(i)=riftheta(i)*exp(h);
dydb(:,i)= (conc(times,theta2,tablet,wt, pma) -
conc(times,riftheta,tablet,wt, pma)) / h ;
end


functiondydz = deriv(times,riftheta,tablet,wt, pma)
dydz = zeros(length(times),length(riftheta));
fori=1:length(riftheta)
h = h=0.00001;*riftheta(i);
theta2 = riftheta;
theta2(i) = riftheta(i) + h ;
dydz(:,i) = (conc(times,theta2,tablet,wt, pma) -
conc(times,riftheta,tablet,wt, pma)) / h ;
end


function y = conc(times,riftheta,tablet,wt, pma)
yo=0;
t =[0 6];
v=riftheta(2)*(wt/12.5);
sol=ode45(@(t,y) rif(t,y,riftheta,tablet,wt, pma), t ,yo);
y=deval(sol,times)/v;
end
```

% Rifampicin model

```
functiondydt = rif(t,y,riftheta,tablet,wt,pma)
MF =1/(1+(pma/58.2).^(-2.21));
cl=riftheta(1)* MF *(wt/12.5)^0.75;
v=riftheta(2)*(wt/12.5);
MTT=riftheta(3);
n=riftheta(4);
F=1;
D=75*tablet;
ka=(n+1)/MTT;
nfac=sqrt(2*3.1415)*(n+1)^(n+1.5)*exp(-n-1);
dydt=(D*F*ka*(ka*t)^(n+1)*exp(-ka*t))/nfac - (cl/v)*y(1);
end
```

% DET pyrazinamide

```
function
finalpyrazoriz=finalDpyraz2cohort(times,pyraztheta,pyrazwmega,pyrazsi
gma,tablet,wt)
oriz=zeros(length(wt));

parfori=1:length(wt)

oriz(i)=finalpyraztwoCohortDfim_with_wt(times,pyraztheta,pyrazwmega,p
yrazsigma,tablet(i),wt(i))
end

finalpyrazoriz=-real(mean(log(-oriz)));

disp(times);
End
```

%TOTALFIM pyrazinamide

```
function
oriz=finalpyraztwoCohortDfim_with_wt(times,pyraztheta,pyrazwmega,pyra
zsigma,tablet,wt)

times1=times(1:4);
times2=times(5:8);
p=0.5;
totalFim1=
pyraztotalFim_with_wt(times1,pyraztheta,pyrazwmega,pyrazsigma,tablet,
wt);
totalFim2=
pyraztotalFim_with_wt(times2,pyraztheta,pyrazwmega,pyrazsigma,tablet,
wt);
totalfim=p*totalFim1 + (1-p)*totalFim2;
oriz =-det(totalfim);
end
```

% FIM pyrazinamide

```
functiontotalFim=
pyraztotalFim_with_wt(times,pyraztheta,pyrazwmega,pyrazsigma,tablet,w
t)
A=pyrazA_with_wt(times,pyraztheta,pyrazwmega,pyrazsigma,tablet,wt);
B=pyrazB_with_wt(times,pyraztheta,pyrazwmega,pyrazsigma,tablet,wt);
totalFim=(1/2)*blkdiag(A,B);
end

function
B=pyrazB_with_wt(times,pyraztheta,pyrazwmega,pyrazsigma,tablet,wt)
dydb=pyrazderivb_with_wt(times,pyraztheta,tablet,wt);
y = pyrazconc_with_wt(times,pyraztheta,tablet,wt);
Var=pyrazVar_with_wt(times,pyraztheta,pyrazwmega,pyrazsigma,tablet,wt
);
Fsasa=trace(Var^-2);
Fsasp=trace((Var^-2)*diag(y.^2));
```

```
Fspsa=trace((Var^-1)*diag(y.^2)*(Var^-1));
Fspsp=trace((Var^-2)*diag(y.^4));
Fp1k=pyrazFp1k_with_wt(times,pyraztheta,pyrazwmega,pyrazsigma,tablet,
wt);
Fjk=(dydb'*(Var^-1)*dydb)^2;
B=[Fjk,Fp1k',Fp1k';Fp1k,Fsasa,Fsasp;Fp1k,Fspsa,Fspsp];
end

function
A=pyrazA_with_wt(times,pyraztheta,pyrazwmega,pyrazsigma,tablet,wt)
dydz = pyrazderiv_with_wt(times,pyraztheta,tablet,wt);
Var=pyrazVar_with_wt(times,pyraztheta,pyrazwmega,pyrazsigma,tablet,wt
);
A=2*dydz'*(inv(Var))*dydz ;
end

function
Fp1k=pyrazFp1k_with_wt(times,pyraztheta,pyrazwmega,pyrazsigma,tablet,
wt)
dydb=pyrazderivb_with_wt(times,pyraztheta,tablet,wt);
Var=pyrazVar_with_wt(times,pyraztheta,pyrazwmega,pyrazsigma,tablet,wt
);
Fp1k=zeros(1,length(pyraztheta)-1);
for k=1:length(pyraztheta)-1
    Fp1k(:,k)=dydb(:,k)'*(Var^(-2))*dydb(:,k);
end

function
Var=pyrazVar_with_wt(times,pyraztheta,pyrazwmega,pyrazsigma,tablet,wt
)
w=pyrazWMEGA_with_wt(pyrazwmega);
s=pyrazS_with_wt(times,pyraztheta,pyrazsigma,tablet,wt);
dydb = pyrazderivb_with_wt(times,pyraztheta,tablet,wt);
Var=dydb*w*dydb' + s;
end


functiondydb=pyrazderivb_with_wt(times,pyraztheta,tablet,wt)
dydb=zeros(length(times),length(pyraztheta)-1);
fori=1:length(pyraztheta)-1
h=0.00001;
theta2=pyraztheta;
theta2(i)=pyraztheta(i)*exp(h);
dydb(:,i)= (pyrazconc_with_wt(times,theta2,tablet,wt) -
pyrazconc_with_wt(times,pyraztheta,tablet,wt)) / h ;
end

functiondydz = pyrazderiv_with_wt(times,pyraztheta,tablet,wt)
dydz = zeros(length(times),length(pyraztheta));
fori=1:length(pyraztheta)
h = 0.00001*pyraztheta(i);
theta2 = pyraztheta;
theta2(i) = pyraztheta(i) + h ;
dydz(:,i) = (pyrazconc_with_wt(times,theta2,tablet,wt) -
pyrazconc_with_wt(times,pyraztheta,tablet,wt)) / h ;
end
```

```
function w=pyrazWMEGA_with_wt(pyrazwmega)
w=diag(pyrazwmega.^2);
end


function s=pyrazS_with_wt(times,pyraztheta,pyrazsigma,tablet,wt)
s=diag(pyrazconc_with_wt(times,pyraztheta,tablet,wt)*pyrazsigma(1).^2
+ pyrazsigma(2).^2);
end

function y = pyrazconc_with_wt(times,pyraztheta,tablet,wt)
yo=[0 0];
t =[0 6];
v=pyraztheta(2)*(wt/12.5);
sol=ode45(@(t,y) pyraz_with_wt(t,y,pyraztheta,tablet,wt), t ,yo);
amount=deval(sol,times);
y=amount(2,:)/v;
end
```

%Pyrazinamide model

```
functiondydt = pyraz_with_wt(t,y,pyraztheta,tablet,wt)
dydt=zeros(2,1);
cl=pyraztheta(1)*(wt/12.5).^0.75;
v=pyraztheta(2)*(wt/12.5);
ka=pyraztheta(3);
MTT=pyraztheta(4);
n=pyraztheta(5);
F=1;
D=150*tablet;
ktr=(n+1)/MTT;
nfac=sqrt(2*3.1415)*(n)^(n+0.5)*exp(-n);
dydt(1)=(D*F*ktr*(ktr*t)^(n)*exp(-ktr*t))/nfac - ka*y(1);
dydt(2)=ka*y(1)-(cl/v)*y(2);
end
```

%DET isoniazid

```
function
finalisonoriz=finalDison2cohort(times,isontheta,isonwmega,isonsigma,a
cetfactor,ffactor,tablet,wt,pma)
oriz=zeros(length(wt));
parfori=1:length(wt)
oriz(i)=finalisontwoCohortDfim(times,isontheta,isonwmega,isonsigma,ac
etfactor(i),ffactor(i),tablet(i),wt(i),pma(i))
end

finalisonoriz=-real(mean(log(-oriz)));
disp(finalisonoriz);
disp(times);
end
```

%TOTALFIM isoniazid

```
function
oriz=finalisontwoCohortDfim(times,isontheta,isonwmega,isonsigma,acetf
actor,ffactor,tablet,wt,pma)
times1=times(1:4);
times2=times(5:8);
p=0.5;
totalFim1=isontotalFim(times1,isontheta,isonwmega,isonsigma,acetfacto
r,ffactor,tablet,wt,pma);
totalFim2=isontotalFim(times2,isontheta,isonwmega,isonsigma,acetfacto
r,ffactor,tablet,wt,pma);
totalfim=p*totalFim1 +(1-p)*totalFim2 ;
oriz=-det(totalfim);
end
```

%FIM isoniazid

```
functiontotalFim=
isontotalFim(times,isontheta,isonwmega,isonsigma,acetfactor,ffactor,t
ablet,wt,pma)
A=isonA(times,isontheta,isonwmega,isonsigma,acetfactor,ffactor,tablet
,wt,pma);
B=isonB(times,isontheta,isonwmega,isonsigma,acetfactor,ffactor,tablet
,wt,pma);
totalFim=(1/2)*blkdiag(A,B);
end

function
A=isonA(times,isontheta,isonwmega,isonsigma,acetfactor,ffactor,tablet
,wt,pma)
dydz = isonderiv(times,isontheta,acetfactor,ffactor,tablet,wt,pma);
Var=isonVar(times,isontheta,isonwmega,isonsigma,acetfactor,ffactor,ta
blet,wt,pma);
A=2*dydz'*(inv(Var))*dydz ;
end
```

```
function
B=isonB(times,isontheta,isonwmega,isonsigma,acetfactor,ffactor,tablet
,wt,pma)
dydb=isonderivb(times,isontheta,acetfactor,ffactor,tablet,wt,pma);
y = isonconc(times,isontheta,acetfactor,ffactor,tablet,wt,pma);
Var=isonVar(times,isontheta,isonwmega,isonsigma,acetfactor,ffactor,ta
blet,wt,pma);
Fspsp=trace((Var^-2)*diag(y.^4));
Fp1k=isonFp1k(times,isontheta,isonwmega,isonsigma,acetfactor,ffactor,
tablet,wt,pma);
Fjk=(dydb'*(Var^-1)*dydb)^2;
B=[Fjk,Fp1k';Fp1k,Fspsp];
end
```

```
function
Fp1k=isonFp1k(times,isontheta,isonwmega,isonsigma,acetfactor,ffactor,
tablet,wt,pma)
dydb=isonderivb(times,isontheta,acetfactor,ffactor,tablet,wt,pma);
Var=isonVar(times,isontheta,isonwmega,isonsigma,acetfactor,ffactor,ta
blet,wt,pma);
Fp1k=zeros(1,length(isontheta)-2);
for k=1:length(isontheta)-2
    Fp1k(:,k)=dydb(:,k)'*(Var^(-2))*dydb(:,k);
end


function
Var=isonVar(times,isontheta,isonwmega,isonsigma,acetfactor,ffactor,ta
blet,wt,pma)
w=isonWMEGA(isonwmega);
s=isonS(times,isontheta,isonsigma,acetfactor,ffactor,tablet,wt,pma);
dydb = isonderivb(times,isontheta,acetfactor,ffactor,tablet,wt,pma);
Var=dydb*w*dydb' + s;
end

function
s=isonS(times,isontheta,isonsigma,acetfactor,ffactor,tablet,wt,pma)
s=diag(isonconc(times,isontheta,acetfactor,ffactor,tablet,wt,pma)*iso
nsigma(1).^2);
end

function w=isonWMEGA(isonwmega)
w=diag(isonwmega.^2);
end


function
dydb=isonderivb(times,isontheta,acetfactor,ffactor,tablet,wt,pma)
dydb=zeros(length(times),length(isontheta)-2);
for i=1:length(isontheta)-2
h=0.00001;
theta2=isontheta;
theta2(i)=isontheta(i)*exp(h);
dydb(:,i)= (isonconc(times,theta2,acetfactor,ffactor,tablet,wt,pma) -
isonconc(times,isontheta,acetfactor,ffactor,tablet,wt,pma)) / h ;
end


function
dydz = isonderiv(times,isontheta,acetfactor,ffactor,tablet,wt,pma)
dydz = zeros(length(times),length(isontheta));
for i=1:length(isontheta)
h = 0.00001*isontheta(i);
theta2 = isontheta;
theta2(i) = isontheta(i) + h ;
dydz(:,i) = (isonconc(times,theta2,acetfactor,ffactor,tablet,wt,pma)
- isonconc(times,isontheta,acetfactor,ffactor,tablet,wt,pma)) / h ;
end
```

```
function y =
isonconc(times,isontheta,acetfactor,ffactor,tablet,wt,pma)
yo=[0 0 0];
t =[0 6];
v=isontheta(2)*(wt/12.5);
sol=ode45(@(t,y)
ison(t,y,isontheta,acetfactor,ffactor,tablet,wt,pma), t ,yo);
amount=deval(sol,times);
y=amount(2,:)/v;
end
```

%Isoniazid model

```
functiondydt = ison(t,y,isontheta,acetfactor,ffactor,tablet,wt,pma)
dydt=zeros(3,1);
MF =1/(1+(pma/49).^(-2.19));
cl=isontheta(1)* acetfactor * MF *(wt/12.5)^0.75;
v=isontheta(2)*(wt/12.5);
ka=isontheta(3);
MTT=isontheta(4);
n=4;
Q=isontheta(5)*(wt/12.5)^0.75;
vper=isontheta(6)*(wt/12.5);
F=ffactor;
D=50*tablet;
ktr=(n+1)/MTT;
k12=Q/v;
k21=k12*v/vper;
nfac=sqrt(2*3.1415)*(n)^(n+0.5)*exp(-n);
dydt(1)=(D*F*ktr*(ktr*t)^(n)*exp(-ktr*t))/nfac - ka*y(1);
dydt(2)=ka*y(1)- k12*y(2)+ k21*y(3) -(cl/v)*y(2);
dydt(3)=k12*y(2)-k21*y(3);
end
```