PHARMACOKINETICS OF TWICE-DAILY VERSUS ONCE-DAILY DOSING WITH GRANULAR SLOW-RELEASE PARA-AMINOSALICYLIC ACID IN ADULTS ON SECOND-LINE ANTI-TUBERCULOSIS AND ANTIRETROVIRAL TREATMENT

by

Lizanne de Kock, BScMedScHons

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Supervisors:

Prof B Rosenkranz
Prof AH Diacon

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Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my original work and that I have not previously submitted it, in its entirety or in part, at any university for a degree.

Signature:……. **Date**: ….....

Abstract

Background

Para-aminosalicylic acid (PAS) is one of the first effective anti-tuberculosis agents and has become one of the principal second-line drugs to treat patients with an extended resistance spectrum. Despite being one of the oldest anti-tuberculosis drugs, little data is available regarding its pharmacokinetics, drug interactions, genetic factors and dosing regimens, especially for the relative new granular slow release PAS (GSR-PAS) preparation.

Objectives

The aim of the study was to investigate the pharmacokinetics, tolerability and safety of a single 8 g once- or 4 g twice-daily GSR-PAS dose in a multidrug- or extensively drug resistant tuberculosis (M/XDR-TB) population, in which some subjects were also co-infected with the human immunodeficiency virus (HIV). An additional objective was to investigate the potential covariates (i.e. genetic factors and drug interactions) that can alter the pharmacokinetics of PAS.

Study design and methodology

A randomised, two-period, open-label cross-over study was conducted in 32 adults (≥18 years old) with M/XDR-TB admitted at Brooklyn Chest Hospital, Cape Town, South Africa and treated for drug resistant tuberculosis with a multidrug regimen containing GSR-PAS. The subjects were randomised to follow a single 8 g once-daily GSR-PAS regimen or a 4 g twice-daily GSR-PAS regimen for 8 days. On the eighth day blood samples were obtained at 0, 1, 2, 3, 4, 6, 8, 12 and 24 hours. After the 24-hour sample (Day 9) the regimens were crossed-over. The tolerability and safety of the two regimens were determined using Visual Analogue Scales and interviews. PAS plasma concentrations were determined by a developed HPLC-MS/MS method. N-acetyltransferase (*NAT1* and *NAT2*) genotyping was performed. The data of this study together with unpublished data of a previous study in a very similar population were used in a pharmacometric analysis to determine the PK parameters and any subject covariates.

Results and Discussion

In comparison to the 4 g twice-daily GSR-PAS dose, the single 8 g once-daily GSR-PAS dose generated a pharmacokinetic profile with a significantly higher maximum concentration (C_{max}), concentration at 12 hours (C_{12}) and area under the curve 0 to

12 hours (AUC_{12}). The concentrations of all subjects on the twice-daily regimen were maintained above a minimum inhibitory concentration (MIC) throughout a 12-hour interval, while the single 8 g dose was able to sustain the PAS plasma concentrations above the MIC in 18 out of 29 subjects (62.1%) for the entire 24-hour dosing interval. Both regimens were reasonably well tolerated but most subjects preferred the twice-daily dosing. The clearance of PAS was increased by 45% in HIV positive subjects prescribed antiretroviral treatment (ART), possibly due to interaction with efavirenz (EFV). No significant associations were found for any of the individual *NAT1* or *NAT2* genotypes, but a difference between mean concentrations of the different genotypic groups was reported.

Conclusions

The 8 g once-daily dose has the potential to be included in future regimens. The higher peak concentrations achieved can be expected to increase the bactericidal effect of GSR-PAS without significant loss of bacteriostatic effect, i.e. time over MIC. The 8 g once-daily dose has a reasonable tolerability and is potentially easier to supervise in an outpatient setting. Since antiretrovirals (ARVs) increase PAS clearance and decrease PAS exposure in HIV co-infected subjects on ART, the 8 g once-daily dose will be less suitable for maintaining bacteriostasis in these patients (inadequate PAS exposure). Therefore, PAS and ARV interactions need to be clarified before the 8 g once-daily dose can be recommended for the HIV co-infected patients on ART.

Abstrak

Agtergrond

Para-aminosalisielsuur (PAS) is een van die eerste effektiewe anti-tuberkulose middels en het een van die hoof tweede-lyn middels geword om pasiënte met 'n uitgebreide weerstand spektrum te behandel. Ondanks die feit dat PAS die oudste anti-tuberkulose middel is, is daar baie min data beskikbaar met betrekking tot die farmakokinetika, middel interaksies, genetiese faktore en dosering, veral in die geval van die relatiewe nuwe granulêre stadige vrystelbare PAS voorbereiding (GSV-PAS).

Doel

Die doel van die studie was om navorsing te doen oor die farmakokinetika, verdraagsaamheid en veiligheid van ’n enkele 8 g een keer daaglikse en 4 g twee keer daaglikse GSV-PAS dosering in ’n multi- of uitgebreide weerstandige tuberkulose (M/XDR-TB) populasie, waar sommige proefpersone ook met die Menslike Immuniteitsgebreksvirus (MIV) geko-infekteer is. ’n Verdere doel van die studie was om te bepaal of potensiële kovariate soos genetika en medisyne interaksies die farmakokinetika van PAS verander.

Metodes

’n Onwillekeurige, twee-periode, oop-etiket oorkruisingstudie was op 32 M/XDR-TB volwassenes (≥ 18 jaar oud) uitgevoer terwyl hulle vir middel weerstandige tuberkulose in Brooklyn Chest hospitaal (Kaapstad, Suid-Afrika) behandel is. Die deelnemers was onwillekeurig ingedeel om ’n 8 g eenkeer daaglikse GSV-PAS dosering of ’n 4 g twee keer daaglikse GSV-PAS dosering vir agt dae te volg. Op die agste dag was bloedmonsters op die volgende ure 0, 1, 2, 3, 4, 6, 8, 12, en 24 geneem. Na die 24-uur monster (Dag 9) was die doserings omgekeer. Die verdraagsaamheid en veiligheid van die twee doserings is bepaal deur gebruik te maak van Visueel Analogiese Skale en onderhoude. PAS plasma konsentrasies is bepaal deur 'n ontwikkelde HPLC-MS/MS metode. N-asetieltransferase (*NAT1* en *NAT2*) genotipering is uitgevoer. Die data van hierdie studie saam met ongepubliseerde data van 'n vorige studie is gebruik in farmakometriese analise om die farmakokinetiese parameters en enige kovariate te bepaal.

Resultate en Bespreking

In vergelyking met die 4 g GSV-PAS twee keer daaglikse dosis, het die enkele 8 g daaglikse dosis, ‘n pharmakokinetiese profiel met ‘n beduidende hoër maksimum konsentrasie (C_{max}), 12-uur konsentrasie (C_{12}) en area onder die kurwe van 0 tot 12 uur (AUC_{12}), gegenereer.

Die PAS plasma konsentrasies van alle proefpersone, wat op die twee keer daaglikse dosis was, was tydens die 12-uur interval bo die die minimum inhiberende konsentrasie (MIK) gehou. Terwyl die enkele 8 g dosis die PAS plasma konsentrasies vir die duur van die 24 uur interval bo die MIK in 18 van 29 (62%) proefpersone gehandhaaf het. Die meeste proefpersone het die twee-daaglikse dosering verkies, maar beide doserings was redelik goed verdra. Die verwydering van PAS het met 45% toegeneem in HIV positiewe proefpersone wat antiretrovirale behandeling ontvang het, moontlik weens interaksies met efavirenz. Geen beduidende assosiasies vir enige van die individuele *NAT1* of *NAT2* genotiepes was gevind nie, maar ‘n verskil tussen die gemiddelde konsentrasies van die verskillende genotiepes is gerapporteer.

Gevolgtrekking

Die 8 g een keer daaglikse dosis het die potensiaal om in toekomstige doserings ingesluit te word. Die hoër piek konsentrasies van die 8 g daaglikse dosis, kan moontlik die bakterisidiese (kiem-dodende) effek van GSV-PAS verhoog, sonder om die beduidende bakteriostatiese (kiem-inhiberende) effek (o.a. tyd oor MIK), te verloor. Die 8 g een keer daaglikse dosis is redelik verdraagsaam en kan potensieël makliker gekontrolleer word in die geval van buite-pasiënte. Serdert antiretrovirale middels (ARVs) PAS verwydering verhoog en gevolglik die PAS plasma konsentrasies verlaag in die MIV ko-infekteerde proefpersone wat op ARVs is, sal die 8 g een keer daaglikse dosis minder geskik wees vir die handhawing van bakteriostasis in hierdie pasiënte (onvoldoende PAS blootstelling). Dus moet daar klarigheid verkry word oor PAS en ARV interaksies voordat die 8 g een keer daaglikse dosis vir MIV ko-infekteerde pasiënte op ARVs aanbeveel kan word.

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'Learn from yesterday, live for today, hope for tomorrow.
The important thing is not to stop questioning' ~ Albert Einstein

List of Abbreviations and Symbols

<	Less than
≥	More or equal
µg/ml	Microgram per millilitre
ADME	Absorption, Distribution, Metabolism, Excretion
AIDS	Acquired immunodeficiency syndrome
Am	Amikacin
Amx/Clv	Amoxicillin/clavulanate
Anti-TB	Anti-tuberculosis
APAS	Acetyl-p-aminosalicylate
ART	Antiretroviral therapy
ARV	Antiretroviral
AUC	Area under the plasma concentration-time curve
Azr	Azithromycin
Bp	Base pair
C_0	Concentration at time zero
CAF	Central Analytical Facility
Cfz	Clofazimine
Clr	Clarithromycin
Cm	Capreomycin
C_{max}	Maximum observed concentration
C_{min}	Minimum observed concentration
CNS	Central nervous system
Cs	Cycloserine
CSF	Cerebrospinal fluid
CV	Coefficient of variation
CYP	Cytochrome P450
DDI	Drug-drug interaction
DNA	Deoxyribonucleic acid
DOT	Directly observed treatment
DR	Drug -resistant
DR-TB	Drug resistant tuberculosis
DST	Drug susceptibility test

E	Ethambutol
EDTA	Ethylenediaminetetraacetic acid
Eto	Ethionamide
FA	Formic acid
FDA	Food and Drug Administration
G	Gram or relative centrifugal force
Gfx	Gatifloxacin
GSR-PAS	Granular slow-release para-aminosalicylic acid
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
INH	Isoniazid
IS	Internal standard
Km	Kanamycin
Lvx	Levofloxacin
Lzd	Linezolid
m/z	Mass-to-charge ratio
MDR	Multi-drug resistant
MDR-TB	Multi-drug resistant tuberculosis
M/XDR	Multi or Extensively drug-resistant
M/XDR-TB	Multi or Extensively drug-resistant tuberculosis
Mfx	Moxifloxacin
Mg	Milligram
MIC	Minimum inhibitory concentrations
Ml	Millilitre
mM	millimol
MS	Mass spectrometer
Msec	millisecond
NAT	N-acetyltransferase
NHLS	National Health Laboratory Service
Nm	nanometres
Ofx	Ofloxacin
PABA	Para-amino benzoic acid
PAS	Para-aminosalicylic acid
PASU	P-aminosalicyluric acid
PK	Pharmacokinetics

Pto	Prothionamide
QC	Quality control
Rpm	Revolutions per minute
SD	Standard deviation
SDS	Sodium dodecyl sulphate
TB	Tuberculosis
TBE	Tris-Borate-EDTA
Th	Thioacetazone
T_{max}	Time at which C_{max} occurred
Trd	Terizidone
UGT	Uridine diphosphate-glucuronosyltransferase
USA	United States of America
VAS	Visual Analogue Scale
Vm	Viomycin
WHO	World Health Organisation
XDR	Extensively drug-resistant
XDR-TB	Extensively drug-resistant tuberculosis
Z	Pyrazinamide

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Chapter One:
Introduction and Rationale

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis* and is a leading cause of morbidity and mortality worldwide [1, 2]. This global epidemic accounts for approximately 8.8 million (range: 8.5-9.2 million) cases and 1.45 million deaths in which 0.35 million deaths were among HIV positive patients[3]. Although effective anti-tuberculosis (anti-TB) treatment is available, resistant strains of *M. tuberculosis* have emerged [4]. The World Health Organisation (WHO) [5] has estimated that 17% of 9.2 million new cases of active TB had some form of drug-resistant tuberculosis (DR-TB), in which 3.1% were multi-drug resistant (MDR). Approximately, 4.9% of MDR cases are characterised by extensively drug-resistant tuberculosis (XDR-TB), which is associated with incredibly high mortality rates, especially when co-infected with HIV [1,4,6].

Treatment of drug-resistant tuberculosis is difficult and involves complex multiple drug regimens, which often includes second-line drug such para-aminosalicylic acid [1,4]. Para-aminosalicylic acid (PAS) is one of the first anti-bacterial chemotherapy agents used to treat TB and has become one of the principal second-line drugs to treat patients with an extended resistance spectrum [7,8,9]. Despite being one of the oldest anti-TB drugs, little data is available regarding the pharmacokinetics, drug interactions and dosing regimens, especially for the relatively new slow/delayed release preparation.

The dosing regimens for PAS are relatively diverse, with multiple daily dosing being the preferable regimen, although it has never been demonstrated that twice or three times daily dosing provides better results than once-daily dosing. According to the WHO and *the guidelines for the programmatic management of drug-resistant tuberculosis* [10], PAS administration must rely on a balance between its tolerance in the subject and the resources available to observe doses. In resource-poor settings, however, complex dosing procedures are not practical as there is notably poor patient compliance and the need for directly observed treatment (DOT). An ideal treatment regimen in this setting is clearly one in which medications are taken only once-daily.

In addition, patient management is complicated by the treatment of both TB and AIDS, increasing the potential for drug-drug interactions [11]. Only a few interactions between PAS and other medications such as isoniazid (INH) have been reported [12,13]. The current knowledge of metabolic pathways and screening methods for interactions were not available when older drugs were developed [14]. Therefore, the mechanisms for interactions between PAS and other TB treatments, especially the newer medications and with antiretroviral drugs have not yet been investigated or reported.

Besides the drug interactions, several other co-factors/covariates (i.e. genetics, weight, age, and disease) can also alter an individual's drug response. Inter-and intraindividual variability in drug response is a major problem in clinical practice and drug development. Genetic factors are often described as one of these co-factors. Several studies [15, 16,17,18] have investigated the genetic variants and enzyme activity of N-acetyltransferase, the enzyme involved in the acetylation of PAS. However, only a few studies [19,20,21] investigated the influence of genetic variations on PAS plasma concentrations.

The present study provided a comprehensive overview of the pharmacokinetics and tolerability of a single 8 g once-daily or 4 g twice-daily granular slow release PAS dose in an M/XDR-TB population, in which some subjects were also co-infected with HIV/AIDS. Furthermore, the factors (i.e. covariates such as genetics, drug interactions age, weight, disease) that may influence the pharmacokinetics of PAS were investigated.

Since pharmacokinetics is an important element in the study; the literature review provided a general overview of the pharmacological concepts of antimicrobial agents; the co-factors (i.e. patient variability) that alter the pharmacology of a drug; and the clinical aspects of PAS. The first part of the literature review focused on DR-TB and treatment. The next section reviewed the general aspects of pharmacokinetics/pharmacodynamics, dosing, and the influencing co-factors of antimicrobial agents. Finally all the clinical aspects of PAS were summarized.

Chapter Two:
Literature Review

2.1. Burden of drug resistant tuberculosis

TB has afflicted mankind for thousands of years and was believed to be virtually incurable until about 70 years ago, when the first monotherapy [a] anti-TB drugs, streptomycin and PAS, showed promising results. The introduction of these drugs into therapy was followed by the development of a rapid succession of anti-TB drugs including rifampicin, INH, pyrazinamide and ethambutol. The availability of a large number of drugs has become increasingly important following the appearance of drug resistant strains after a few months of treatment [22]. In order to greatly reduce the emergence of resistant strains, a combination of two or more drugs was introduced into the recommended treatment regimens [23].

Poor patient compliance to these multidrug regimens has led to the emergence of a global epidemic of multi-drug resistant TB (MDR-TB). MDR-TB is caused by *M. tuberculosis* strains that are resistant to the first-line TB drugs - rifampicin and INH [1,24]. More recently, the emergence of XDR-TB has been seen worldwide. Here the *M. tuberculosis* strains are additionally resistant to second-line drugs such as the fluoroquinolones and the aminoglycosides - amikacin, capreomycin or kanamycin [10].

Despite the fact that the global TB incidence has slightly decreased, morbidity and mortality resulting from TB in Africa remains unacceptably high [3,25, 26]. During 2006, a total of 464 patients were diagnosed (by NHLS) with XDR-TB in South Africa and increased to 741 patients in 2010 [10]. TB/HIV co-infection is one of the largest contributing factors for the high prevalence of TB in this part of the world [25]. Since the first described outbreak of XDR-TB in patients co-infected with HIV (XDR-TB/HIV) in KwaZulu-Natal Province, South Africa, these most resistant isolates have been diagnosed in 49 countries [6,25,27]. This outbreak has increased the concerns in South Africa with its high prevalence of HIV, as it is likely to lead to an epidemic [25]. In 2006, a report was published from a rural sub-district in KwaZulu-Natal (KZN), showing that

[a] Monotherapy: treatment of a condition by means of a single drug
(Dorland's Medical Dictionary of Health Consumers. Elseriver Inc. 2007)

221 (41%) of 542 TB patients had MDR-TB and 53 (9.7%) of these were XDR-TB patients [28]. All XDR-TB patients were sero-positive for HIV and had a median survival of 16 days after specimen collection [28]. Delayed results of drug-susceptibility testing (DST), which took several weeks meant that appropriate drug therapy could not be administered early enough.

XDR-TB is difficult to treat in both HIV positive and negative patients, and has a significantly worse treatment outcome than MDR-TB [29]. Various factors contribute to this outcome, but drug interactions and resistance to the most potent anti-TB drugs are often the main issues.

2.1.1. Treatment of Drug resistant Tuberculosis

Drug resistant TB is treated with second-line drugs, which are much more expensive, less effective and have more side effects than first-line drugs [1,14]. As with drug susceptible TB, the use of a combination of several antibiotics is imperative to prevent additional resistance [10]. If the patient with MDR-TB has a history of using second-line drugs, an individualised regimen based on the DST results is essential for designing such a regimen. In addition, patients with resistance to a fluoroquinolone and an injectable drug will also require drugs such as capreomycin, PAS granules, terizidone, moxifloxacin or levofloxacin, high dose INH and clofazimine among other drugs in their regimens. In the case of XDR-TB, individualized treatment regimens are equally essential in order to design a suitable regimen [10,30]. Table 1 summarises the different groups of anti-TB drugs used in drug-resistant TB. According to the *Management of Drug-Resistant Tuberculosis: South Africa Policy Guidelines, 2011* [10], the recommended standardised regimen for MDR and XDR-TB in South Africa is as follows, but this may be modified based on DST results:

- MDR-TB; 6 months: kanamycin/amikacin-moxifloxacin-ethionamide-terizidone-pyrazinamide/ 18 months: moxifloxacin-ethionamide-terizidone-pyrazinamide
- XDR-TB; 6 months: capreomycin-moxifloxacin-ethionamide-terizidone–pyrazinamide-PAS-clofazimine/ 18 months: moxifloxacin-ethionamide-terizidone or cycloserine-pyrazinamide–PAS/clofazimine

Table 1 Grouping of DR-TB drugs

Group	Drugs
Group 1: First-line oral drugs	Ethambutol (E) Pyrazinamide (Z)
Group 2: Injectable drugs	Kanamycin (Km) Amikacin (Am) Capreomycin (Cm) Viomycin (Vm)
Group 3:Fluoroquinolones	Levofloxacin (Lvx) Moxifloxacin (Mfx) Gatifloxacin (Gfx) Ofloxacin (Ofx)
Group 4: Oral bacteriostatic second-line drugs	Ethionamide (Eto) Prothionamide (Pto) Cycloserine (Cs) Terizidone (Trd) Para-aminosalicylic acid (PAS)
Group 5: Drugs of unclear efficacy (Not recommended for routine use in MDR-TB subjects)	Clofazimine (Cfz) Amoxicillin/clavulanate (Amx/Clv) Clarithromycin (Clr) Azithromycin (Azr) Linezolid (Lzd) Thioacetazone (Th) High-dose isoniazid (INH) Imipenem

(Adapted from: WHO [30])

The desired properties of an anti-TB agent are to rapidly eliminate metabolising bacilli, sterilize TB lesions and prevent resistance to companion drugs [2,31]. The actions of anti-microbial drugs are dependent on the dynamic combination of the host, microbe and drug factors [31]. This relationship is often studied in pharmacokinetics and pharmacodynamics.

Pharmacokinetics involves the dose-concentration relationship, while pharmacodynamics governs the concentration-effect relationship or the clinical effect of a drug [32].

2.1.2. Pharmacokinetics (PK)

Pharmacokinetics (PK) is a quantitative study of the time a drug is absorbed, distributed, metabolised and excreted (ADME) [2,33,34]. Each process of ADME is associated with one or two parameter(s) that are dependent on a drug, its metabolites and the subject [35].

A pharmacokinetic profile of a drug can be developed when plasma concentrations are plotted over time. With this drug profile several pharmacokinetic parameters can be calculated using mathematical models [33]. These parameters include maximum

and minimum concentrations (C_{max} and C_{min}), time to reach the maximum concentration (t_{max}), area under the curve (AUC), bioavailability (F), clearance (CL), half-life, or volume of distribution (V_d); and can be used to calculate optimum drug dosages for an individual or a group of individuals.

2.1.3. Pharmacodynamics (PD)

Pharmacodynamics is the study of how a drug acts on a living organism, including the pharmacologic response and the duration and magnitude of response observed relative to the concentration of the drug at an active site in the organism. The pharmacodynamics of an anti-tubercular agent describes the relationship of the drug concentration to the pharmacologic effects of a drug that enables eradication of the pathogen [32,36]. Based on anti-microbial pharmacodynamic properties an antimicrobial can be classified as bactericidal or bacteriostatic. Bactericidal agents such as INH will rapidly decrease the colony forming units in the sputum of TB patients after starting the treatment [31].

In contrast to the bactericidal group, the bacteriostatic agents such as PAS, ethionamide and terizidone inhibit the growth of bacilli [32,37,38]. This group requires the assistance of the host defence mechanisms to clear the tissue of bacilli. If the host defences mechanisms are inadequate to remove the microbe, it will resume growth after discontinuing of the bacteriostatic agent [39]. Therefore, a combination of bacteriostatic and bactericidal agents is prescribed in TB regimens.

The antimicrobial activity of an antimicrobial agent can be determined *in vivo* or *in vitro*. The early bactericidal activity (EBA) of a drug is the decreasing rate of colony forming units (CFU/ml) in a patient's sputum during the first two days of drug administration and is expressed as $\log_{10}$ (CFU/ml)/day. A drug's sterilizing activity is the rapid sterilization of the sputum and prevention of relapse [2]. In contrast to EBA and sterilizing activity, the minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MBC) can quantify the drug potency *in* vitro [2,39,40]. The MIC is the lowest drug concentration that inhibits microbial growth after incubation of 16-20 hours with a standard inoculum ($1\text{-}5x10^5$ CFU/ml). The MBC is the drug concentration that kills 99.99% of the initial inoculum [2].

The concentration of the bacteriostatic agents must exceed the MIC to actively inhibit the pathogen. Isolates of microbe species can have variable MICs. Resistant isolates have relative high MICs, while sensitive isolates have low MICs [39,41]. The MBC of a bacteriostatic agent is many-fold higher than its MIC, while a bactericidal agent's MBC is usually the same or less than four-fold of its MIC [39].

Bactericidal agents can be subdivided as either time-dependent bactericidal activity (concentration independent) or concentration-dependent bactericidal activity. For drugs with time-dependent bactericidal activity such as beta-lactam agents, the concentration must exceed the MIC for the pathogen. Killing of the bacteria depends on the time that the bacteria are exposed to the drug. The rate of eradication does not increase even if concentration increases beyond four to five times that of the MIC [38,39,41,42]. The clinical success is based on the percentage of time that the drug concentration at the site of action is above the MIC (T>MIC) [32]. Therefore, the duration of exposure needs to be optimised based on the correlation between the time above the MIC, and the bacteriologic and clinical outcome [37].

For antibiotics with concentration-dependent bactericidal activity, such as rifampicin, INH, aminoglycosides and fluoroquinolones, a sufficient maximum concentration at the site of infection is required. The eradication rate increases over a wide range of concentrations [32,39]. The C_{max} to MIC and/or the AUC to MIC ratios correlate best with the eradication rate [32, 38,40]. In contrast to a time-dependent agent, this group of drugs has a more significant post-antibiotic effect. This is the phenomenon whereby there is a maintained suppression of the bacterial growth after exposure to the drug, even when the concentrations are below the MIC [32,37,38].

2.1.4. PK/PD and dosing of anti-TB agents

The purpose of therapeutics is to achieve a desired beneficial effect with minimal adverse effects. A rational approach in determining the appropriate drug and dose for a certain disease such as TB is to combine the principles of pharmacodynamics and pharmacokinetics, and to clarify the dose-effect relationship[43]. Both pharmacokinetics and pharmacodynamics are involved in the determination of dosing regimens of antimicrobial agents [37]. Figure 1 illustrates the relationship between pharmacokinetics and pharmacodynamics for antimicrobials. The pharmacokinetics of a drug

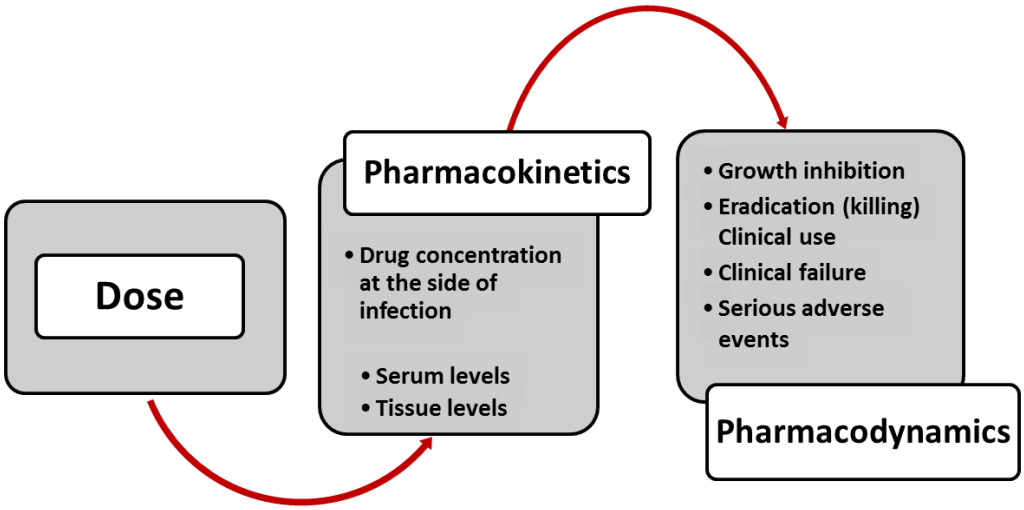

Figure 1 Inter-relationship of pharmacokinetics and pharmacodynamics of antimicrobial agents [32]

determines the drug concentration in serum and tissues over time whilst the pharmacodynamic parameters integrate the antimicrobial activity [32].

In antimicrobial therapy, complex interactions between the pathogen, the individual and the drug are inevitable. In clinical situations, the complexity is usually reflected by high variability in the dose-response relationship [42]. To reduce the variability in drug and dose response, the pathogen, the host and the pharmacological properties of the drug need to be taken into account when determining the ideal drug and dose to be used in a clinical situation [40,42].

The PD/PK parameters that correlate with clinical outcome are different for time-dependent and concentration-dependent antimicrobials, and therefore dosing strategies are also different [32]. For the time-dependent and bacteriostatic drugs, the dosing strategies should maximise the time the drug concentration is above the MIC at the site of action. For the concentration–dependent drugs, the highest possible peak concentration is preferable to maximise the effect of the drug [38]. However, high concentrations can be toxic to the host. For example, high doses of fluoroquinolones can result in CNS toxicity [32,44]. Each drug has a specific therapeutic range, where the drug concentration is considered safe and effective in the general population. Figure 2 illustrates the relationship between the efficacy and toxicity of a drug. The adjustable elements of dose, dose frequency, dosage form and route of

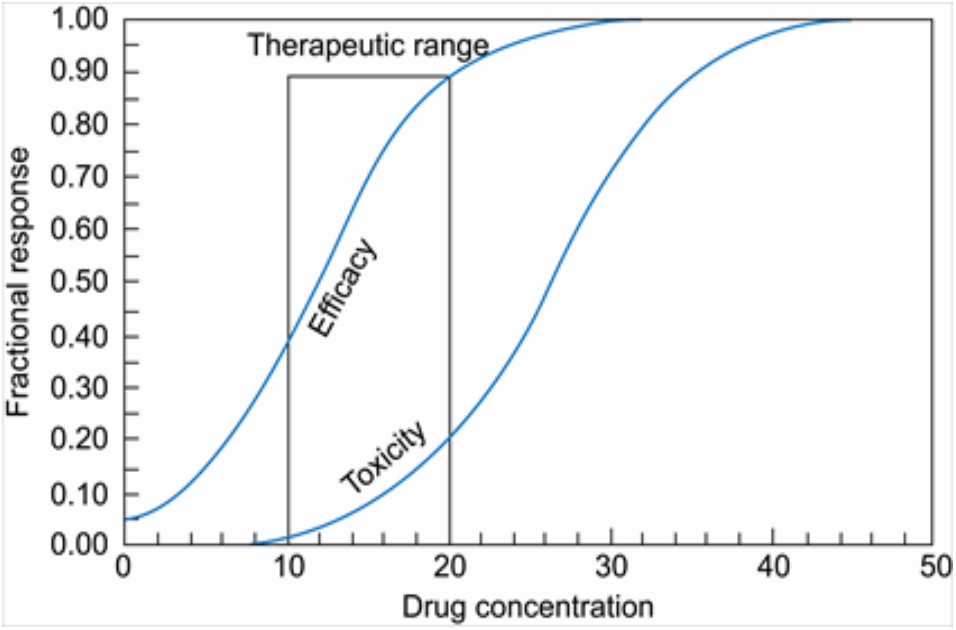

Figure 2 Therapeutic range of a drug [medicinescomplete.com]

administration are directly related to toxicity or therapy failure [45]. For example if the dosage exceeds the drug removal rate, the drug will accumulate and can reach toxic levels [45]. Accumulation of the drug is often seen after several doses of the drug. If the dose is too low, the levels will fall below the therapeutic range and the drug will fail to work.

Intraindividual/interoccasion[b] and interindividual[c] variability of pharmacokinetics is often a result of variability in the ADME of the drugs, for example as a result of the patient's general state of health, organ dysfunction, or genetic factors [35].

2.2. Factors influencing the PK/PD profile of a drug

The ideal dose of a drug is the one that is clinically effective and results in no adverse effects. Unfortunately, medication is seldom both safe and effective in all subjects [46]. Interindividual variability in drug response is a major problem in the clinical practice and drug development. It often leads to therapeutic failure of drugs or adverse events in individuals from various sub-populations [47]. Mehta et al. [48] found

[b] Intraindividual/interoccasion: variation within an individual
[c] Interindividual: variation within a population

that adverse drug reactions contribute significantly to morbidity and patient hospitalization in South Africa. The adverse drug reactions rate of 14% occurring in their study population was double that reported in a systematic review of international studies.

Several factors can be involved in the large variation observed in interindividual drug response and includes the patient's age, body weight, gender, genetic factors, renal and liver function, disease, drug-drug interactions, and lifestyle such as smoking and alcohol.

2.2.1. Age

The aging process can significantly affect the drug pharmacokinetics due to impairment in the function of many regulatory processes[49]. The reduced gastrointestinal blood flow and higher gastric pH in elderly affects the extent of absorption of the drug. The physical changes in aging such as muscle atrophy and increased body fat can also affect the volume of distribution. Drug elimination is affected by decreased kidney function (decrease in renal plasma flow and glomerular filtration resulting in decrease of drug excretion) and reduced enzymatic capacity (reduced metabolism) in the elderly [49,50,51].

Furthermore, elderly patients often use more drugs, therefore increasing the potential of drug-drug interactions [52].

2.2.2. Body weight

Patients with different body weights given similar doses will produce different drug plasma concentrations. A large body weight is associated with a larger volume of distribution and clearance, especially for highly lipophilic drugs which may result in lower drug plasma concentrations [53]. In the case of an obese patient the drug distribution will be affected due to more fat. Therefore, adjustment of the dose may be necessary in such cases. Decreased body weight and wasting are more relevant in TB-patients, e.g. with wasting, the albumin and leptin concentrations will be reduced, affecting the protein binding of drugs [54]. Dose adjustment of drugs should therefore be considered in patients with large increase or decrease in body weight.

2.2.3. Diseases

Diseases cause physiological and pathological changes, which can change the drug pharmacokinetics. Absorption, distribution and elimination can be altered by diseases affecting the organ responsible for these functions. For example, patients with congestive heart failure have reduced cardiac output and liver blood flow, which will decrease the hepatic drug elimination [35]. Another example is the high drug concentrations observed in patients suffering from renal disease. The clearance of the drug or metabolites through the kidneys decreases in patients with renal dysfunction, and the compounds will accumulate in the body.

Infection such as empyema or abscess can also affect drug activity by decreasing the pH or reduce the oxygen content at the site of action consequently reducing the activity of certain drugs such as aminoglycosides [40].

2.2.4. Genetic factors (Pharmacogenetics)

Pharmacogenetic/genomics is a growing field in industry and has become an important element in drug and dose design. This field explores the interactions of drugs with genetically determined effectors and metabolic pathways, and has an influence on drug safety, drug efficacy and disease progression [55].

Forty years ago researchers realized that some adverse drug reactions and inefficacy could be a result of genetically determined variations in enzyme activity [56, 57]. Many of these variations were identified by the occurrence of adverse reactions after standard doses of the drugs in healthy volunteers or patients [58]. Treatment failure in individuals and their family members confirmed that genetic variations in drug metabolising enzymes were the cause of these outcomes [56, 57].

There are several types of genetic variations that have a direct or indirect effect on drug response and development of various diseases. Variations in the genes encoding any of the proteins involved in the ADME of a drug can alter the drug response and pathological outcome.

Drug transporters expressed in the brain, intestine, liver and kidney play an essential role in the ADME of several drugs. Genetic heterogeneity on drug transporters can

contribute to interindividual variation in drug disposition and drug response [55]. The transporter, P-glycoprotein (P-pg) functions as a transmembrane efflux pump and will move drug molecules from intracellular to extracellular domain [55]. Therefore, variations in the gene encoding this protein will have an indirect effect on drug availability, since the protein controls the efflux of drugs in the cellular domain.

Genetic polymorphisms have been described for several phase I (cytochrome P450 isoforms) and phase II (N-acetyltransferase, uridine diphosphate-glucuronosyltransferase) drug-metabolising enzymes, and can influence the plasma concentrations of the drugs that are a substrate for these enzymes. For example, genetic variants in N-acetyltransferase 2 (*NAT2*) gene alters the patient's ability to acetylate INH and will result in toxic levels of the drug, which can lead to peripheral neuropathy or hepatotoxicity [59,60]. Another well-known clinical example is the CYP2B6 polymorphism which is well defined as an important predictor of the efavirenz (EFV) pharmacokinetic parameters [61].

It was noted that pharmacogenetic variations occur at different frequencies in the population, especially in a population with different ethnic groups. For example, the *NAT2* divides the population into homozygous fast, heterozygous fast and homozygous slow *NAT2* acetylators of INH [17,59]. Another example is the variation in CYP2B6 (*CYP2B6*6*) that is more common in African-Americans compared to Caucasians [55]. The CYP2B6*6 variant is associated with an increased liver toxicity; hence more African-Americans than Caucasians will develop liver toxicity [62]. Therefore, ethnic origin and population differences have to be considered in pharmacotherapy [59].

Variable drug response and efficacy reflects the combined influence of environment, gender, disease, concomitant drugs, nutrition and genetic factors [55,59]. The individual's enzymes together with the other co-factors and drug pharmacology will therefore determine the individual's drug response [46].

Environmental compounds and different drugs can alter gene expression by interacting with nuclear receptors such as CAR, PXR, PPAR and AhR [59]. The CYP group of enzymes is often induced or inhibited by various compounds and drugs, even if these chemicals are not a substrate for the specific isoform. The following section describes the influence of drug interactions on drug response and PK.

2.2.5. Drug interactions

Polypharmacy is essential in the treatment of tuberculosis, especially in DR-TB. Several drugs are used in order to prevent resistance to certain susceptible drugs. However with the use of multiple drugs, the risk for potential drug-drug interactions increases. In addition to DR-TB, it is common to treat several other concurrent health conditions such as HIV/AIDS or non-communicable diseases such as diabetes or hypertension increasing the potential for drug interactions. Drug interactions are defined as a modification of the effect of a drug when co-administered with another drug. This effect may enhance or reduce the action of either drug which can result in possible over- or under-dosing and lead to a lack of drug efficacy or toxicity [63]. Drug interactions can be divided in three groups: pharmacodynamic, pharmacokinetic or combined interactions [64,65,66].

2.2.5.1. Pharmacodynamic interactions

Pharmacodynamic interactions amongst others occur when the effect of a drug is changed by another drug at the site of actions, without changing the plasma concentrations of either drug. It arises from an antagonistic (drug effect is decreased), additive or synergistic effect (drug effect is increased), and may alter the pharmacological response, such as toxicity and efficacy.

Antagonism of antimicrobial agents occurs when the combined killing effect or inhibitory effects of two or more antimicrobial drugs are significantly less than the activity of individual drugs. For example, rifampin can antagonise vancomycin action against staphylococci [67].

Additive/synergistic effect occurs when the activity or toxicity of a drug is enhanced due to the presence of another drug with a similar pharmacological action. An example is the great delay in development of resistance to streptomycin when co-administered with PAS [68] .

2.2.5.2. Pharmacokinetic interactions

Pharmacokinetic interactions are the most common interactions [66]. Interactions of this kind may either alter the drug plasma concentration and/or its tissue distribution,

consequently affecting the concentration or availability of the drug to the target site.

These particular interactions occur when absorption, distribution, metabolism and/or excretion of a drug is altered [63,64,69].

2.2.5.2.1. Drug interactions associated with absorption

Absorption is the movement of compounds into the circulatory system from site of administration (e.g. gastrointestinal, skin, subcutaneous site, nasal/pulmonary sites, and muscles). The absorption of drugs can be altered by several physiological factors such as intestinal motility, gastric emptying time, gastric pH, transport, intestinal metabolism, and the presence of gastrointestinal disease [69]. In addition, concomitant substances such as drugs, can also affect the drug absorption by having a large surface area upon which the drug can be absorbed; alternating the gastric pH; binding or chelating; altering gastrointestinal motility or affecting the transport proteins [61,63,65.]

The most common interaction affecting drug absorption is chelating, which is the binding of metal ions or other substances to the drugs in the gastrointestinal tract [61]. For example, PAS absorption is decrease due to the binding of divalent cations (iron, magnesium and calcium) to PAS molecules [70].

2.2.5.2.2. Drug interactions associated with distribution

The important mechanisms by which drug interactions can alter drug distribution are the competition for protein binding; displacement from tissue binding sites and alterations of the local tissue barriers [65]. The competition of the drugs for protein binding affects the degree of the free drug (pharmacologically active form). Theoretically the plasma concentration of the free drug will increase, but in this case the concentration is maintained due to an increase in free drug elimination [63]. Therefore, protein binding displacements are usually considered to be of less clinical significance [69,71].

2.2.5.2.3. Drug interactions associated with drug metabolism

Co-administered drugs may directly or indirectly alter the concentration and activity of another drug by inhibiting or inducing the enzyme activity responsible for the metabolism of the drug.

Several clinically important drug interactions have been reported. Some drug interactions can be beneficial, by increasing the activity or bio variability of an effective drug. However, this can also result in high drug levels, which can be toxic and life-threatening [45,61]. For example, co-administration of PAS and INH can be beneficial, but can also result in severe neurotoxicity and/or hepatotoxicity. PAS inhibits the acetylation of INH, consequently increasing the INH levels [72].

Both phase I and II enzyme activities can be altered by their substrate or other compounds. Even the transporters such as P-pg are susceptible to drug induction or inhibition [61].

The CYP enzyme group consisted out of 12 isoforms and are the major enzyme group involved in the drug metabolism [73]. Several anti-TB and ARV drugs are metabolised by the CYP group and therefore are susceptible to drug interactions. Some of these drugs are also inducers and inhibitors of the CYP group, which complicates the design of regimens for DR-TB subjects that are also co-infected by HIV/AIDS [61]. Alterations in the plasma levels of an antimicrobial or antiretroviral agent can cause sub-therapeutic drug concentrations, which can result in developing of pathogen drug resistance. In risk patients the plasma levels should be monitored to ensure that toxicity is reduced and the MIC is exceeded [45]. The Clinical Pharmacology Division of Indiana University summarised the clinically relevant drug interactions of the CYPs. This can be found at:

http://medicine.iupui.edu/clinpharm/DDIs/ClinicalTable.aspx.

2.2.5.3. Combined toxicity

The use of combinations of drugs for a prolonged period, as in TB treatment, often results in drug toxicity [38]. Several drugs have similar toxicities which can lead to

increased toxicity when co-administered (shared toxicity). In combining these drugs in a regimen, the potential of organ damage is increased. For example, ethionamide and PAS have toxic effects on the thyroid. These drugs are often prescribed in a regimen and long term use can lead to hypothyroidism. In some cases, a drug(s) can enhance the organ toxicity of another drug, even if the enhancing drug has no intrinsic toxicity effect on the organ [65].

DR-TB patients often develop hepatotoxicity and renal dysfunction since multiple drugs including more toxic second-line drugs, are prescribed to these patients. These drugs are often metabolised in the liver and excreted by the kidneys. Therefore, monitoring of liver and kidney functions are important in these patients [10].

2.3. Para-aminosalicylic acid (PAS)

Para-aminosalicylic acid (PAS, p-aminosalicylic acid, 4-aminosalicylic acid, 4-ASA), is a second-line anti-TB agent that has been found to be effective in preventing drug-resistance and has formed part of the standard treatment for TB for several decades[6]. However, due to its significant gastrointestinal intolerance, it was replaced by ethambutol, an equivalent drug in terms of efficacy, but with a better tolerability profile[11]. Due to the relatively low use of PAS over the last three decades, most isolates of TB remained susceptible to this drug [11;14]. Therefore, PAS was brought back in clinical use for the management of MDR-TB and later XDR-TB.

A new granular slow-release PAS formulation (GSR-PAS) with fewer gastrointestinal adverse effects was developed which supported the use of PAS as one of the principal second-line drugs in M/XDR-TB treatments[8]. Currently, the GSR-PAS (PASER® granules, Para-aminosalicylic Acid Delayed-Release Granules-JACOBUS Pharmaceutical Company Inc., Princeton, NJ 08540) is the only form available in most countries including South Africa.

2.3.1. Indications

GSR-PAS (PASER) is classified as a tuberculostatic agent. It is mainly used to treat drug resistant tuberculosis (M/XDR-TB) or in cases where therapy with INH and rifampicin is not possible due to intolerance and/or a combination of resistance. PAS

is prescribed together with other anti-TB agents to which the specific pathogen strain of the individual patient is expected to be susceptible [12].

2.3.2. Mechanism of action

PAS is a highly specific bacteriostatic agent against *M. tuberculosis* and valuable in preventing resistance to other drugs such as INH and streptomycin [9,12]. It has a molecular weight (M_w) of 153.14 (Figure 3) and inhibits the growth of *M. tuberculosis in vitro* at a concentration of 1-2 µg/ml [2].

The mechanism of PAS is still unclear, but it has an influence on the folate pathway of bacteria. Bacteria use folate derivatives as co-factors in the biosynthesis of important molecules such as amino acids, thymidylate, pyrimidines and purines[2]. Bacteria are unable to use external sources of folic acid; therefore a deficiency in these important molecules inhibits bacterial growth.

PAS is structurally similar to sulphonamides (Figure 4), which is an analogue of para-amino benzoic acid (PABA) (Figure 5). PABA is a substrate for dihydropteroate synthase (DHPS), which is an important enzyme in folate biosynthesis of bacteria [8;74]. PAS has therefore been considered to interfere with bacterial DHPS by functioning as a competitive inhibitor (competing with PABA). However, PAS inhibitory activity of *folP1* (encodes DHPS) appears to be poor *in vitro* [75] (Figure 6).

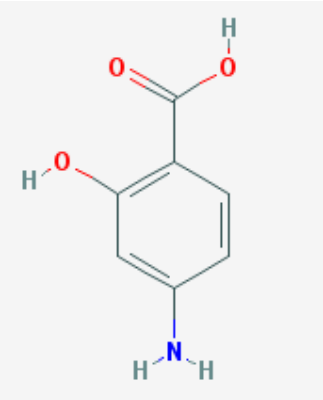

Figure 3 Structural formula of PAS

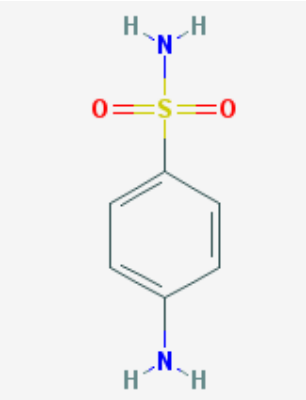

Figure 4 Structural formula of sulphonamides

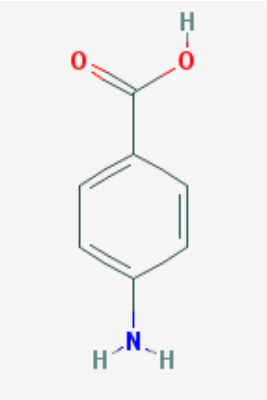

Figure 5 Structural formula of PABA

A mutation in the thy A gene of clinical PAS-resistant isolates (PAS^r) was identified and found to be related to a mechanism for drug resistance to PAS [8] (Figure 6). The thy A gene encodes thymidylate synthase A, which is required for thymine biosynthesis in the bacterial folate pathway. This finding implies that PAS acts as a folate antagonist which inhibits folic acid synthesis, but without potentiating anti-folic compounds [8].

A recent study [76] investigated the mechanism of action of PAS and reported that PAS, in contrast to the initial speculation, serves as a replacement substrate for DHPS and not as a competitive inhibitor. The PAS metabolites and the subsequent steps in folate metabolism inhibit the enzymes and compete with their substrates. According to the authors, PAS is a pro-drug that blocks the growth of *M. tuberculosis* when its active forms are generated by enzymes in the pathway, which functions as a poison to the bacteria.

PAS is also thought to inhibit the synthesis of the cell wall component, mycobactin, which decreases iron uptake of the bacteria [2,13].

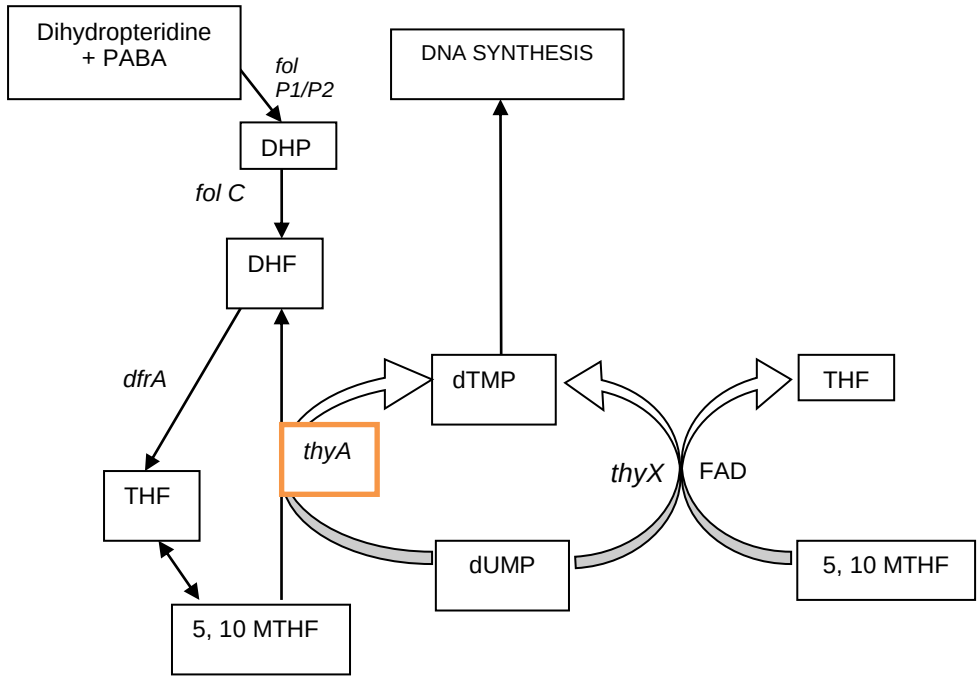

Figure 6 Postulated mechanism of the action of PAS [Modified from Rengarajan[8] and Mathys[74]

2.3.3. Pharmacokinetics of PAS

With GSR-PAS preparation, the PAS is encapsulated in an acid resistant enteric coating to prevent early breakdown in the stomach. Once the GSR-PAS formulation reaches the more alkaline smaller intestine, it dissolves and is readily absorbed. It is therefore recommended to be taken with an acidic beverage or food, enhancing the delayed-release properties of the GSR-PAS [12]. The rate of drug absorption depends on the rate of release from the formulation [35]. With the controlled-release formula, the drug is released at a constant rate over a longer period and fluctuations in the drug plasma concentrations are reduced in comparison to the immediate-release formulations such as the sodium-PAS.

PAS is well absorbed from the gastrointestinal tract. A plasma concentration of 2 µg/ml is reached within 2 hours after a 4 g GSR-PAS dose. In a study in healthy volunteers a mean maximum concentration (C_{max}) of 20 µg/ml (range: 9-35 µg/ml) with a mean peak time of 6 hours (range: 1.5-24 hours) was achieved after a single 4 g GSR-PAS dose [12,77,].

In a study in twelve MDR-TB patients a mean C_{max} of 51.3 µg/ml (range: 25.8-93.1 µg/ml) and a mean peak time of 5.2 hours (range: 0-8 hours) was achieved after a 4 g GSR-PAS dose [78].

PAS is 50-60% protein bound and is well distributed throughout the body. It is only detected in the cerebrospinal fluid (CSF) if the meninges are inflamed (10-50% of the plasma levels) [2,12,77]. PAS has a plasma half-life ($t_{½}$) of 1-2 hours. It is rapidly metabolised in the intestinal mucosa and liver through acetylation by N-acetyltransferase 1 (*NAT1*), in which more than 50-70% of the drug is acetylated to N-acetyl-para-aminosalicylic acid (APAS) [2, 11, 77, 79]. Twenty five present (25%) of the absorbed PAS dose is conjugated to form p-aminosalicyluric acid (PASU) [79]. Although PAS is the active form, it is reported that PASU has a 75% inhibitory effect in comparison to APAS which shows little, if any tuberculostatic affect [80].

The drug and its metabolites are excreted by tubular secretion and glomerular filtration. Approximately 80% of the drug is excreted in the urine, with about 50% is in the acetylated form. PAS plasma concentrations are not significantly changed by hepatic or renal insufficiency, but with severe renal insufficiency the PAS and its metabolite will accumulate [77].

2.3.4. PAS dosing regimens

The current prescription for GSR-PAS is 8-12 g in divided doses[10]. Since PAS is bacteriostatic, several daily doses are recommended in order to maintain the concentration of PAS above the MIC of 1 µg/ml [76,81]. It was noted that divided doses reduce the intolerance to PAS [81]. However, it has been reported in several early studies that once-daily dosing of PAS was better tolerated than multiple dosing [80,82,83].

Early studies experimented with various doses of PAS in combination with other drugs such as INH. A study [84] in 1957 found that dosage of 10-12 mg/kg PAS in divided doses accompanied by INH provided better results than 5-6 mg/kg PAS which was also accompanied by INH, implying that higher concentrations of PAS will enhance the drug activity.

Clinical [82] and animal [85] studies have investigated the possibility of decreasing the frequency of PAS administration to a single daily dose. Karlson and Carr [85] experimented with a 125 mg single daily sodium PAS dose in guinea pigs and reported that the once-daily dose was as effective as the twice-daily dose, despite the fact that PAS is rapidly absorbed and excreted.

Bridge and Carr [82] investigated the effect of a single 5 g dose of PAS combined with 150 mg of INH given twice-daily to 70 subjects. They compared these results to 192 subjects that followed a regimen of 3 g PAS three times a day. In the 5 g daily group negative sputum cultures were reported in nine subjects (12.9%) after two months and in five subjects (7.1%) after four months. Thirty one patients (16.1%) of the 3 g PAS three times daily group discontinued the PAS treatment due to intolerance, while only three subjects (4%) who used the single 5 g dose developed intolerance. According to Bridge and Carr [82], the once-daily dosing was not only better tolerated as multiple doses, but equally effective.

Bang et al.[86] investigated various PAS preparations in single and divided doses and concluded that divided doses of PAS give lower PAS concentrations. Although multiple doses still maintain the PAS concentration above the MIC, a single daily dose is considered to be a more rational treatment because it increases the free PAS levels in the plasma.

PAS was previously available as potassium, sodium or calcium salts and as an acid

preparation. Sodium salt preparations, which are still marketed, are completely and rapidly absorbed, and a peak is seen within 90-120 minutes after drug administration [13,79]. Since PAS has a short half-life, more frequent dosing is required, especially when using the salt formulations. The salt formulations are not as well tolerated as the GSR-PAS preparation.

Not only is the GSR-PAS preparation better tolerated, but maximum concentrations of PAS are seen after 4-6 hours [12,13]. Therefore, the drug is longer present in the systemic circulation, and less frequent dosing is needed to maintain the PAS concentration above the MIC.

Only one study [81] investigated different dosing strategies with the GSR-PAS preparation. The investigators compared a single 4 g once-daily and 4 g twice-daily GSR-PAS dose to determine if a once-daily dosing regimen will be sufficient. PAS concentrations were maintained above the MIC throughout the twice-daily dosing interval, but not with the once-daily regimen. The granules were generally well tolerated in this study; however a few subjects reported nausea and gastrointestinal discomfort.

2.3.5. Resistance

M. tuberculosis isolates have developed resistance to various antibiotics and antimicrobial chemotherapy agents, including PAS [8]. However, resistance to PAS has been reported to be less common than to other antibiotics. Since PAS is a bacteriostatic agent, it is important to maintain the plasma concentration above the MIC in order to inhibit bacteria growth and the development of resistance as discussed above.

The MIC of PAS is different among the various multi-drug resistant strains. The MIC for *M. tuberculosis* in 7H11 agar was less than 1.0 µg/ml for nine strains including three multidrug resistant strains tested, but 4 and 8 µg/ml was measured for two other multidrug resistant strains. Little dose response was seen with the 90% inhibition in 7H12 broth (Bactec), but the MIC was interpreted as being less than or equal to 0.12-0.25 µg/ml for eight strains[12].Therefore, a MIC of 1 µg/ml can be seen as the natural MIC of *M. tuberculosis* for PAS.

2.3.6. Tolerability and safety

The most common side effects of PAS are hypothyroidism and gastrointestinal intolerance which includes nausea, vomiting, abdominal pain, diarrhoea and anorexia [12,13,77]. Prolonged administration of PAS often results in hypothyroidism and is increased with the co-administration of ethionamide.

Hepatitis has been reported in 0.3-0.5% of patients receiving PAS. In a review of 7,492 patients treated with PAS, 0.5% developed hepatitis[12]. This condition emerges within three months of therapy, is often associated with rash and fever, and less frequently with anorexia, nausea or diarrhoea[12]. In a few days or weeks, 90% of the patients show symptoms of jaundice. Hepatitis is usually accompanied by hepatomegaly, and often by leucocytosis, lymphadenopathy, and eosinophilia [87]

Other rare side effects include leukopenia, hypoprothrombinemia, agranulocytosis, goitre, thrombocytopenia, Coomb's positive haemolytic anaemia, and lupus-like syndrome. The side effects of PAS are summarised in Table 2 [12, 87]. More information regarding the side effects is available in Appendix A: PASER package insert.

Table 2 Reported side effects of PAS

Disorder	**Side effects**
Blood dyscrasias and lymphatic systems disorders	Leucopoenia, agranulocytosis, thrombocytopenia, eosinophilia, Coombs positive haemolytic anaemia may develop in patients with G6PD deficiency
Cardiovascular	Pericarditis
Coagulopathy	Hypoprothrombinaemia has been reported.
Dermatological side effects	Skin rash, erythematous maculopapular and pruritic lesions Exfoliative dermatitis
Dysglycaemia	Hypoglycaemia has been reported

Table 2 Reported side effects of PAS

Disorder	Side effects
Endocrine effects	Hypothyroidism and goitre relatively common
'Flu-like' syndrome	Löffler's syndrome, an infectious mononucleosis-or lymphoma-like syndrome, has been reported
Gastrointestinal disorders	Nausea, vomiting, abdominal pain Diarrhoea Rarely, associated with peptic ulcers gastric haemorrhage
General disorders	Fever; thrombocytopenia
Hepatobiliary disorders	Jaundice, hepatitis
Hypersensitivity	Lymphadenopathy, leucocytosis, conjunctivitis, headache, joint pain, vasculitis,
Metabolism and nutrition disorders	Malabsorption syndrome, including: steatorrhea, an abnormal small bowel pattern on X-ray, villus atrophy, reduced cholesterol, D-xylose and iron absorption. Malabsorption of vitamin B12, folate, iron and lipids may results in clinically important erythrocyte abnormalities
Nervous system disorders	Optic neuritis, encephalopathy, psychosis
Renal and urinary disorders	Hypocalcaemia
Respiratory, thoracic and mediastinal disorders	Eosinophilic pneumonia
Vascular disorders	Vasculitis

2.3.7. Pharmacogenetics of PAS

The N-acetyltransferase 1 (*NAT1*) is the only gene that has been studied in the pharmacogenetics of PAS, since it is predominantly involved in PAS metabolism.[2,79,88]. N-acetyltransferase (NAT) enzymes are involved in the metabolism of various drugs such as PAS, INH and dapsone. Belonging to the phase II enzymes, they are important in the detoxification of harmful chemicals, and in deactivation and elimination of certain drugs. This group of enzymes catalyse the N-acetylation and O-acetylation of heterocyclic amine, aromatic amine and various aryl amine and hydrazine drugs[15,89]. Both genes of the isoforms (*NAT1* and *NAT2*) are located on the short arm of chromosome 8 (*NAT*18p21.3-23.1 and *NAT2* 8p21.3-23.1 and 8p22). These genes are 87% similar on the nucleotide level, which translates to an 81% homology at an amino acid level [90].

Both *NAT1* and *NAT2* are known to be polymorphic. *NAT2* is more extensively studied than *NAT1*, due to its clinical relevance in the metabolism of INH [15]. Initially it has been believed that *NAT1* is monomorphic, with no distinct differences in its phenotype or genetics[91]. However, it was soon discovered that this assumption is incorrect. Studies have identified *NAT1* allelic variants that are associated with increased enzyme activity[92]. Several oncology studies [89,93] have also reported the association of *NAT1* polymorphisms with cancer development, especially bladder and breast cancers. It seems that *NAT1* and *NAT2* polymorphisms can interact with each other and increase or decrease the risk for cancer development [93].

Several distinct allelic polymorphisms at the *NAT1* locus cause a large variability in the human population [58]. So far, 26 alleles have been described [94]. The *NAT1*3*, *NAT1*4*, *NAT1*5*, *NAT1*10* and *NAT1*11* are the most common alleles. The allelic variations are single polymorphisms or a combination of a number of nucleotide substitutions and insertions/deletions, which will increase (e.g. *NAT1*10*, *NAT1*21*, *NAT1*24* and *NAT1*25*) or decrease (*NAT1*14*, *NAT1*15*, *NAT1*17*, *NAT1*19* and *NAT1*22*) or have no effect (e.g. *NAT1*11*, *NAT1*20* and *NAT1*23*) on acetylation activity with respect to enzyme encoded by the *NAT1*4* (wild type[d]) [20,94,95]. The *NAT1*11* allele has been considered as a rapid allele in Caucasians and Black South Africans [96].

[d] The normal, non-mutated version of a gene common in nature

*NAT1*4* has been found in higher frequency among Caucasian populations than in Asian and African populations, whilst the opposite may be true for the *NAT1*10* [15,95]. The *NAT1*3* allele is found in a higher frequency among Chinese than in Caucasian or African Americans [97].

2.3.8. Drug interactions of PAS

Only a few drug interactions with PAS have been described. One of the most important interactions is the decrease in INH acetylation. PAS administered as a 12 g dose has been reported to reduce the acetylation of INH by 20%, especially in rapid acetylators in the population [98].

PAS reduces the absorption of vitamin B_{12}, folate, lipids, rifampicin, digoxin and iron. PAS (5 g) can reduce the absorption of vitamin B_{12} by 55%, which can lead to erythrocyte abnormalities [12,87,].

So far no interactions have been described between PAS and any of ARVs[14]. Table 3 summarises the known drug-drug interactions between PAS and other medications.

Table 3 Drug-drug interactions between PAS and other medications

Concomitant drugs	**Effect**
Angiotensin converting enzyme inhibitors	PAS may reduce the antihypertensive effect
Azathioprine	PAS may increase the toxicity
Carbonic anhydrase inhibitors	Potentiate adverse events of both PAS and the inhibitors
Corticosteroids	Increase the adverse events of corticosteroids
Digoxin	PAS decreases absorption with 20%
Diphenhydramine	Impaired absorption of PAS
Ethionamide	Potentially increased risk of hepatotoxicity
Folate, lipids, iron.	PAS reduces the absorption of folate, lipids, iron

Table 3 Drug-drug interactions between PAS and other medications

Concomitant drugs	Effect
INH	Inhibited acetylation of INH and may lead or increase concentrations
Loop diuretics	PAS may reduce the effect of loop diuretics, and the loop diuretics can increase the serum levels of PAS
Mercaptopurine	PAS may increase the toxicity
Methotrexate	PAS may increase toxicity
Non-selective NSAIDS (except diclofenac)	Increase adverse effects of PAS
Oral anticoagulants, thrombolytics or salicylates	PAS may increase the risk of bleeding
Probenecid	Competitive excretion: increased PAS levels
Rifampicin	PAS reduces the absorption of rifampicin
Sulfonylurea	PAS may increase the hypoglycaemic effect
Sulindac Vitamin B12	PAS may decrease the serum concentration PAS decrease absorption of vitamin B12
Systemic corticosteroids	Increase the number and severity of adverse effects, especially gastrointestinal
Thioguanine	PAS may increase the toxicity
Tolmetin	PAS may increase the risk of GI bleeding
Treprostinil	PAS may increase the risk of bleeding

Adapted from http://www.drugbank.ca/drugs/DB00233, Mllleron [87], Arbex et al.[13]

Chapter Three:
Study Objectives

3.1. Primary objective:

The primary objective of the study was to investigate the pharmacokinetics of GSR-PAS given 8 g once-daily vs. 4 g twice-daily and to establish whether once-daily dosing results in concentrations above the MIC of *M. tuberculosis* for a period comparable to twice-daily dosing in adult M/XDR-TB subjects.

3.2. Secondary objectives:

- To investigate the safety and tolerability of PAS when given once-daily vs. twice-daily

- To investigate the pharmacokinetics of PAS in subjects on antiretroviral treatment and those not on antiretroviral treatment

- To investigate the pharmacokinetic effects of genetic variations of the genes involved in PAS metabolism (*NAT1* and *NAT2*)

Chapter Four:
Materials and Methods

Unpublished data of the pharmacokinetic profile of GSR-PAS given twice-daily were available from a previous project performed by the Division of Pharmacology (samples collected partly by Dr A Liwa) and by a team of researchers under Prof A Diacon in Brooklyn Chest Hospital. These data were analysed and used as a pilot study to plan the main study comparing once vs. twice-daily GSR-PAS (study outlined in the title of the dissertation). The pilot study will be referred to as study 1 and the main study as study 2.

Both studies were conducted according to the ethical guidelines and principles of the International Declaration of Helsinki, South Africa Guidelines for Good Clinical Practice and the Medical Research Council Ethical Guidelines for Research. Both had received ethical approval from the Health Research Ethics Committee at the University of Stellenbosch (study 1: N09/08/212; study 2: M12/01/006), the Western Cape Department of Health and the director of the hospital. The final approved versions of the protocols for studies 1[e] and 2 are given in Appendix B and C.

4.1. Study 1: Pharmacokinetic profile of GSR-PAS given twice-daily

The aim of the study was to describe the pharmacokinetic profile of GSR-PAS (PASER® granules, Para-aminosalicylic Acid Delayed-Release Granules-JACOBUS Pharmaceutical Company Inc., Princeton, NJ 08540) given twice-daily. The data of all subjects in the study were documented for non-compartmental pharmacokinetics analysis and for population pharmacokinetic evaluation (see pharmacokinetic analysis and population pharmacokinetic sections).

Forty one adults hospitalised at Brooklyn Chest Hospital, Cape Town, South Africa participated in the study in 2010 and 2011. Written informed consent for the study

[e] It is important to note that the protocol of study 1 in Appendix B is the original protocol of Dr Liwa. His study mainly focused on the pharmacokinetics of PAS in children; the adults were used as the reference group. As extension of this study, the pharmacokinetics of PAS was investigated in adults receiving the drug as twice-daily doses of 4 g on two separate occasions.

and separately for genotyping was obtained from each subject after all study procedures, risks and benefits had clearly been explained in their preferred language (Appendix D).

The first twelve subjects received their 12-hourly 4 g GSR-PAS dose a day before and on the pharmacokinetic blood sampling day (6:00 and 18:00 hours). The blood samples were scheduled to be collected at 0, 2, 3, 4, 5, 6, 8 and 12 hours. The 12 hour blood sample was taken before the subjects received their second daily dose.

The following twenty nine subjects also received GSR-PAS (4 g) twice-daily, but the second dose was given at 16h00. The blood samples were scheduled to be collected at 0, 2, 3, 4, 5, 6, 8 and 12 hours.

In both groups the pharmacokinetic blood sampling was repeated after two to four weeks. All relevant information was recorded on a case report form (CRF) (See Appendix E).

4.2. Study 2: Pharmacokinetic profile of once-daily vs. twice-daily GSR-PAS

4.2.1. Study design and subjects

Adults (≥18 years old) admitted and treated for DR-TB at Brooklyn Chest Hospital, Cape Town, South Africa were enrolled. Written informed consent for the study and separately for genotyping was obtained from each subject after all study procedures, risks and benefits had clearly been explained in their preferred language. The subject information and consent forms were available in English, Afrikaans and IsiXhosa (Appendix F).

Subjects were enrolled into the study if they were stable on an unchanged TB regimen for two weeks, including PAS; and had no concurrent illness that according to the investigator made the participation inadvisable because of potential interference with the study or increased risk to the subject.

The study was a randomized, two-period, open-label cross-over trial, where each subject received two different dosing regimens for GSR-PAS. All subjects had reached pharmacokinetic steady state conditions (>2 weeks treatment duration) for

GSR-PAS. In this cross-over study[f] each subject received PAS orally either as a single daily (24-hourly) 8 g dose (1x 8 g GSR-PAS) or as a twice-daily (12-hourly) 4 g dose (2x 4 g GSR-PAS) on 2 separate occasions. The subjects were assigned to one of the two regimens using a randomisation schedule drawn up by the statistician. The dosing regimen was followed for 8 days and on the eighth day blood samples were collected at 0, 1, 2, 3, 4, 6, 8, 12 and 24 hours. After the 24 hour sample (Day 9) the regimens were crossed-over, therefore subjects who received 2x 4 g GSR-PAS, were switched to 1x 8 g GSR-PAS and vice versa. The study procedures are outlined in Table 4 and Figure 7.

Table 4 Summary of study events

	Days							
Test	**-7 to -1**	**1 to 7**	**8**	**9**	**10 to 15**	**16**	**17 to 22**	**23**
Screening	**X**							
Randomisation		**X**						
Prescribed PAS routine	**X**						**X**	**X**
GSR-PAS 2x 4g*		**X**	**X**	**(X)**	**(X)**	**(X)**		
GSR-PAS 1x 8g*		**(X)**	**(X)**	**X**	**X**	**X**		
PK sampling			**X**			**X**		
Cross over*				**X**				
VAS		**X**	**X**	**(X)**	**(X)**			
Follow up								**X**

(X)-GSR-PAS 1x 8 g*; X-GSR-PAS 2x 4 g*

***Subjects were randomised to start with either treatment and switched to the alternative treatment at Day 9

[f] Cross-over study: The administration of two or more experimental therapies one after the other in a specified or random order to the same group of subjects(www.medicine.ox.ac.uk)

<table>
<tr><td>Days
-7 to -1</td><td>Day
0</td><td>Days
1-7</td><td colspan="2">Day 8</td><td>Day
9- 15</td><td colspan="2">Day 16</td><td>Day
17-23</td></tr>
<tr><td rowspan="4">Screening</td><td rowspan="4">Randomisation</td><td colspan="3">Continuous dosing with PAS during this period</td><td colspan="3">Cross-over; Continuous dosing with PAS during this period</td><td rowspan="4">Follow-up</td></tr>
<tr><td>No PK samples will be taken for 7 days</td><td colspan="2">Blood sampling</td><td>No PK samples will be taken for 7 days</td><td colspan="2">Blood sampling</td></tr>
<tr><td>2x 4 g GSR-PAS</td><td>Subjects taking ARVs</td><td>Subjects not taking ARVs</td><td>1x 8 g GSR-PAS</td><td>Subjects taking ARVs</td><td>Subjects not taking ARVs</td></tr>
<tr><td>1x 8 g GSR-PAS</td><td>Subjects not taking ARVs</td><td>Subjects taking ARVs</td><td>2x 4 g GSR-PAS</td><td>Subjects not taking ARVs</td><td>Subjects taking ARVs</td></tr>
</table>

2x 4 g GSR-PAS: 8 days of dosing with PAS 4 g twice-daily;
1x 8 g GSR-PAS: 8 days of dosing with PAS 8 g once-daily

Figure 7 Schematic illustration of the study procedure

4.2.2. Demographic and medical information

All relevant demographic and medical information; dosing and blood sampling events; as well the concomitant medication were well recorded in three separate case report forms (CRF) at enrolment and at the two sampling days (Appendix G). The weight and height were measured on the sampling days.

4.2.3. Drug administration

GSR-PAS (PASER® granules, Para-aminosalicylic Acid Delayed-Release Granules-JACOBUS Pharmaceutical Company Inc., Princeton, NJ 08540) were prescribed by the attending physicians for all the subjects as part of their multi-drug regimens (See Appendix A for PASER package insert). Concomitant medication was allowed according to the resistance pattern of each subject and to concurrent illnesses.

The subjects were assigned to the following regimens using a randomisation schedule.

2x 4 g GSR-PAS: Twice-daily dosing- Subjects received a 12-hourly 4 g oral GSR-PAS dose with other already prescribed medicines (ARVs, anti-TB drugs and other drugs).

1x 8 g GSR-PAS: Once-daily dosing- Subjects received a single 8 g once-daily oral GSR-PAS dose along with other already prescribed medicines (ARVs, anti-TB drugs and other drugs).

GSR-PAS was given 1-2 hours prior (06h45-07h30) to breakfast and other medications; and the second dose (12-hourly) for the 2x 4 g GSR-PAS was given 1¼ - 2 hours after dinner (18h45-19h30). All doses were taken either with yoghurt (100 g) or amasi[g] (125 ml) under direct observation. These acidic beverages prevent early release of the drug in the stomach [81]. Subjects had the option to take the 8 g GSR-PAS (once-daily) either with one or two 100 g yoghurts or amasi. Each subject's dosing event was recorded on a dosing form (Appendix H).

4.2.4. Tolerability and Safety

Tolerability and safety were assessed by using self-rating Visual Analogue Scales (VAS). These scales were 10-point scales, with the far left indicating no symptoms and far right as severe symptoms (Appendix I). Gastrointestinal intolerance is frequently reported for PAS and is manifested by vomiting, diarrhoea, nausea, bloatedness and abdominal pain; therefore these side effects were included in the VAS.

On a daily basis the subjects were individually interviewed and the scales were thoroughly explained before completion. These activities took place before the second dose (twice-daily), one to two hours after the last meal in order to record the daily food consumption.

On the last blood sampling day, the subjects were asked about their experiences during the study and their response was recorded. A follow-up interview was done after a week.

4.2.5. Sampling days

The sampling days were scheduled to be on Day 8 and Day 16 of the study. Three millilitre blood specimens were collected in EDTA-containing tubes through a

[g] Amasi is the common African word for fermented milk that tastes like cottage cheese or plain yoghurt. This is the renowned drink of the Masai warrior tribes in Northern Tanzania and Kenya. This drink is renowned for its rich variety of beneficial microorganisms and highly bioavailable nutrients (naturalnews).

catheter inserted into a forearm vein at time 0 (pre-dose), 1, 2, 3, 4, 6, 8, 12 (pre-dose, second daily dose) and 24 hours after dosing. The second dose for the twice-daily dosing was scheduled to be immediately after the 12-hourly blood sample.

4.3. Experimental method

4.3.1. Blood samples

All blood samples were collected on ice and centrifuged for 10 minutes at 3500 g. Thereafter, the plasma was promptly harvested and frozen at -80ºC for analysis. The cellular matter was kept in the EDTA tubes for genetic analysis.

4.3.2. Bio analysis of PAS

A HPLC-MS/MS method for the quantitative determination of PAS in plasma was developed by the Division of Pharmacology and applied to analysis the samples.

4.3.2.1. Chemicals and reagents

Pure PAS (A 7,960-4) powder and the internal standard (IS), thiazetazone, were purchased from Sigma-Aldrich (St Louis, USA). Methanol was purchased from Merck (Darmstadt, Germany) and formic acid (FA) from Fluka Chemie GmBH (Buchs, Switzerland).

4.3.2.2. Preparation of calibration curves and quality controls

Pure PAS compound specifications were used for calibrations and development of respective calibration curves. In each sample batch 8-10 calibrators of different concentrations were included in the run as quality control (QC) samples. For the preparation of the calibrators an appropriate amount of the working solutions and the internal standard were added to blank serum samples to achieve a specific range (1.0 to 100 µg/ml.) of calibration concentrations. All stock solutions were stored at -80°C and were thawed to room temperature prior to use.

4.3.2.3. Stability

The stability of PAS in frozen (-80˚C) samples was determined over a time period of six months; no degradation could be detected within this time period. The samples were analysed within 2 weeks after collection.

4.3.2.4. Sample preparation

The frozen plasma samples were first thawed to room temperature prior to the extraction procedure. Once thawed, the samples for HPLC analysis were prepared by pipetting 100 µl of subject plasma and 300 µl of methanol (Merck, Darmstadt, Germany), containing 1.0 µg/ml thiazetazone (IS) (Sigma, St Louis, USA) into a 2 ml test tube in order to precipitate the plasma proteins. The test tube was vortexed for 5 minutes to ensure complete mixing of all substances. Thereafter, the samples were centrifuged at 11 000 g for 5 minutes. Two hundred and fifty micro litres (250 µl) of the supernatant was transferred into suitable auto sampler vials for analysis and was placed in the auto sampler set at 6°C.

4.3.2.5. Chromatographic system

PAS concentrations were determined by a binary high performance liquid chromatography (Agilent Series 1100 HPLC, Agilent Technologies, Waldbronn, Germany) equipped with an Agilent Zorbax analytical column (150 mm x 2.1 mm), 3.5 µm particle size.

Concentrations were determined by means of a gradient method. For elution, a gradient of water with 0.1% formic acid (FA) as the hydrophilic phase (mobile phase A), and methanol with 0.1% FA as the lipophilic phase (mobile phase B) was used. All the solvents were of HPLC grade and were filtered through a 0.45 µm filter to remove possible particular matter. The gradients started with 90% A and 10% B, which was maintained for 95 seconds. Then, it was changed within 5 seconds to 10% A and 90% B, and maintained for 7 minutes. Then, the gradient was changed within 10 seconds to 90% A and 10% B, and maintained for 4.4 minutes before returning to the initial conditions. The flow rate was 300 µl/min and the column temperature at

40˚C. The volume injected from each sample vial was 5 µl and the samples were kept at 6°C.

4.3.2.6. MS/MS Analysis

The concentration of PAS was determined by means of an API 2000 tandem mass spectrometer (MS/MS) (Applied Biosystems, MDS Sciax, Foster City, Canada) equipped with an atmospheric turbulon ionization chamber. A single quantifier transition range for the thiazetazone (IS) with a precursor and product ion of m/z 237.12/119.96 (dwell 500 msec) was used, while a transition of m/z 237.12/134.10 (dwell 500 msec) was used as qualifier. The m/z quantifier transition for PAS was 154.20/136.20 (dwell 500 msec) and the qualifier transitions were 154.2/119.2 and 154.2/108.2 (dwell 500 msec). The quantifier m/z transition was used for quantification, while the indicated qualifier m/z transition was used to ensure selectivity of the compound. Thiazetazone (IS) eluted after 7.0 minutes and PAS after 5.42 minutes. The lowest limit of detection was 0.25 µg/ml. A switching valve was used to only introduce the column effluent into the nebulising chamber (375 ˚C) at specified time ranges to avoid unnecessary contamination of the nebulising chamber. The time range for PAS was from 4.5 to 6.0 minutes and 6.5 to 7.5 minutes for the IS.

4.3.2.7. Accuracy, precision and recovery

The accuracy and intra-day precision of the method were determined by measuring replicate serum samples of the test compound at various concentrations. Intra-day variation was less than 3% while daily variation was found to be less than 5% over the respective calibration range. A variation of more or less 5% was found over the entire duration of the analysis.

4.4. Pharmacokinetic analysis

The non-compartmental pharmacokinetic analysis was utilized to obtain the steady state pharmacokinetic parameters of concentration-time profile for each subject, using the software WinNonLin v5.3 (Pharsight, Mountain View, CA). The following parameters were determined:

C_{max} maximum observed concentration

C_{min} minimum observed concentration

C_{last} last observed concentration

C_0 observed plasma concentration at 0 hours pre-dose

C_{12} observed plasma concentration at 12 hours post-dose

C_{24} observed plasma concentration at 24 hours post-dose

t_{max} time from dosing to when C_{max} was reached.

t_{min} time from dosing to when C_{min} was observed.

t_{last} time from dosing to when C_{last} was observed.

AUC_{last} area under the concentration-time curve from dosing to the last time point with measurable drug concentration. AUC_{last} was calculated by the linear trapezoidal method.

$t_{½}$ apparent plasma half-life of each analyte, calculated as:

$$t_{1/2} = \frac{0.693}{\lambda_z}$$

where λ_z, the apparent first order elimination rate constant, was determined by fitting a log-linear least-square regression to the terminal phase of the plasma time-concentration data; the slope of the terminal phase is the λ_z. At least three time points starting from the last observed concentration was used to define the slope of the terminal phase.

AUC_{∞} area under the plasma concentration-time curve extrapolated to infinity which is calculated by:

$$AUC_{\infty} = AUC_t + \frac{C_{last}}{\lambda_z}$$

where C_{last} was the last measurable concentration.

AUC_{12} area under the concentration-time curve from dosing to the 12 hour post-dose.

AUC_{24} area under the concentration-time curve from dosing to the 24 hour post-dose. If the 24 hour drug concentration is missing, the 24-hour drug concentration is extrapolated from the slope of the regression and the area is computed from the last measurable concentration using linear trapezoidal rule.

CL_{ss}/F apparent total body clearance at steady-state, calculated as:

$$CL_{ss}/_{F} = \frac{Dose}{AUC_{\tau}}$$

V_z/F_{obs} apparent volume of distribution during the terminal phase

4.5. Genotyping

4.5.1. DNA Purification

The cellular matter obtained in the sample preparation process outlined in 4.3.1 was kept in the EDTA tubes and stored at –20˚C until analysed. An aliquot volume of blood was transferred to a sterile 50 ml polypropylene centrifuge tube and diluted with 5-6 volumes of ice-cold Cell Lysis Buffer (1% Triton X-100, 320 mM sucrose, 5 mM $MgCl_2$, 10 mM Tros, pH 7.6) and mixed by 20-30 hand inversion. The tubes were centrifuged at 3000 rpm for 15 minute at 4˚C in an Eppendorf bench top centrifuge (Model 5810R). The cellular pellet obtained was re-suspend in 6 ml of Nuclei Lysis Buffer (400 mM NaCl, 10 mM Tris pH 8.2, 2 mM EDTA pH 8.2) with 1% sodium dodecyl sulphate (SDS) and 200 µg proteinase K enzyme solution (10 mg/ml) and incubated overnight at 37˚C. Two millilitre (2 ml) of saturated NaCl solution (>6 M) was added and gently hand mixed until it was homogeneity. It was then centrifuged at 4000 rpm for 30 minutes (4˚C) to a pellet of degraded proteins. The genomic DNA was precipitated from the supernatant by adding 2, 5 volumes of absolute ethanol (room temperature). Thereafter, the DNA was spooled out with a heat-sealed Pasteur pipette and was washed with 70% ethanol, heat dried for 15-20 minutes at 37˚C and re-dissolved in 800 µl of sterile 0.5 x TE (1 mM EDTA pH 8.0, 10 mM Tris pH 8.0) buffer for 16 hours at 4˚C.

4.5.2. DNA Concentration and Purity

The quantification of purity nucleic acid solution was determined spectrophotometrically at 260 and 280 nanometres (nm), by measuring the absorbance of a diluted sample placed in a beam with a one centimetre path length. A solution of double stranded DNA with a concentration of 50 µg/ml has an A_{260} equal to one. The ratio of the 260 nm and 280 nm readings (A_{260}/ A_{280}), estimate the DNA purity in the solution, and are usually between 1.8 and 2.0, thus representing good deproteinisation of the samples.

The integrity of the genomic DNA was assessed by agarose gel electrophoresis against a commercially supplied preparation of purified lambda DNA (Roche, USA). The DNA appeared as a single high molecular weight band, comparable in size and intensity to the lambda DNA band.

4.5.3. Agarose Gel Electrophoresis

Seakem ® LE agarose (BMA) gels were used for the electrophoresis of the DNA and evaluation of the PCR amplification products. The PCR product sizes were compared to commercially supplied DNA makers, 100 bp DNA ladder (Promega, USA). The gels were prepared in 0.5 x Tris-Borate-EDTA (TBE) buffer (45 mM Tris, 1 mM EDTA pH 8.0, 45 mM Borate) and set in a mini-gel electrophoresis apparatus (Omeg Scientific, South Africa) with gel dimensions of 100 mm x 65 mm x 3.5 mm. The DNA samples were mixed with a Blue/Orange DNA loading dye (Promega, USA), loaded on the gel, and electrophoresed at 15 volt. A 0.5 x TBE was used as the running buffer. The gels were then stained at room temperature for 20 minutes, in a solution of 0.5 x TBE containing the Gelstar® Nucleic Acid stain (BMA), diluted to a 1 x final concentration. The gels were visualized on the UV transilluminator (UVP, USA) and photographed using the Kodak electrophoresis Documentation and Analysis 120 system (Kodak Digital Science, Eastman Kodak Co., USA)

4.5.4. *NAT1*

A 1492 bp domain of the *NAT1* gene, spanning several genetic variations, including twenty-one single nucleotide polymorphisms (SNPs) and a number of inserted and/or

deleted sections within the 3'-UTR of the gene (http://www.louisville.edu/medschool/pharmacology/)[h], was amplified using the gene-specific primers: *NAT1* fwd. (5'-^{117}AGGATACCAGTTGG-3') and *NAT1* rev (5-^{1585}TTCGAAAATTACAACCAAATAACAA-3'). The amplification reaction was carried out in a total volume of 50 µl, using 0.92 units of Phusion® High-Fidelity DNA Polymerase (Finnzymes Oy, Finland). The reaction consisted out of 30 ng genomic DNA, 0.2 mM dNTP mixture (dATP, dGTP, dTTP and dCTP), 10 µl 5x of Phusion® High-Fidelity DNA Polymerase, 0.25 µM of forward and reverse primer, and 10 mM $MgCl_2$. Amplification was performed in a Veriti 96-well Thermal Cycler (Applied Biosystems, USA). The temperature cycling conditions were as follow: initial denaturation at 98ºC for 1 minute; 35 cycles of alternating denaturation at 98ºC, for 15 seconds; annealing at 60ºC for 20 seconds; polymerisation at 72ºC for 35 seconds; and a final polymerisation of 72ºC for 10 minutes.

Three micro litres of the PCR product was then assessed for specificity and efficiency using a submarine gel electrophoresis in 1% Seakem® LE agarose (BMA, USA) gel using 1x Sodium Borate (SB) buffer (5 mM $Na_2B_4O_7.\cdot 10H_2O$), and a current of 250 volts for 15 minutes in the Omeg mini-gel apparatus (Omeg Scientific,RSA). The electrophoresis results were visualised as described above in the 'Agarose Gel Electrophoresis' section.

4.5.4.1. Direct sequencing

The amplified *NAT1* domain (PCR product) was assessed by direct sequencing at the Central Analytical Facility (CAF), Stellenbosch University. All sequencing results were quality controlled by the staff at CAF, and the sequence alignment analysis was performed using Sequencher, version 4.10.1 (http://www.genecodes.com), and the *NAT1* reference sequence (http://www.ncbi.nlm.nih.gov/nuccore/14018355; Genbank Accession Number AJ307007).

4.5.5. *NAT2*

A fragment (1000 bp) of the *NAT2* gene was amplified using the gene specific primer set, supplied by Integrated DNA Technologies (www.idtdna.com): HuNAT14 (5'-

[h] This Human *NAT1* and *NAT2* alleles are listed by an international gene nomenclature committee

^{726}GACATTGAAGCATATTTTGAAAG^{748}-3') and HuNAT16 (5'-^{1724}GATGAAAGTATTTGATGTTTAGG^{1702}-3'). The PCR amplification was performed in a total volume of 100 µl, consisting out of 150 ng genomic DNA, 50 mM KCl. 20 mM Tris buffer (pH 8.4), 0.2 mM dNTP mixture (dATP, dGTP, dTTP and dCTP), 2.5 mM $MgCl_2$, 0.25 µM of the primers, and one unit of *Taq* DNA Polymerase 1 (Gibco BRL Life Technologies, USA). The amplification was performed in the Gene Amp PCR system 2700 (Applied Biosystems, USA). The temperature cycling conditions were as follow: initial denaturation at 94ºC for 5 minute; 35 cycles of alternating denaturation at 94ºC, for 30 seconds; annealing at 56ºC for 40 seconds, polymerisation at 72ºC for 80 seconds, and an extended polymerisation at 72°C for 10 minutes, where after the sample tubes were cooled and maintained at 4°C for 30 minutes.

Five micro litres of the PCR product (*NAT2)* was analysed for efficacy and specificity by submarine gel electrophoresis (Omeg mini gel apparatus) as described in the 'Agarose Gel Electrophoresis' section.

4.5.5.1. Restriction Endonuclease Analysis

Restriction endonuclease analysis was used to determine the genetic variations/polymorphisms of *NAT2 (NAT2*7; NAT2*6 and NAT2*14).* The digestions were performed in accordance with the recommendations of the specific manufacturer. Fifteen micro litres of the PCR product was separately digested with the *Bam HI, Dde I, Fok I, KpnI, MspI,* and *Taq I* restriction endonucleases, respectively. The *Bam HI, KpnI*, and *MspI* restriction products were additional digested by *PstI.* These double digests generated fragment profiles possessing bands of less than 500 bp in size, which are specific for *MspI, KpnI* and *BamHI*, respectively. This provided the accurate resolution of these profiles on a non-denaturing polyacrylamide gel electrophoresis.

In comparison to the above, the amplified *NAT2* gene sequence possesses several cutting sides for the *Dde I, Fok I,* and *Taq* restriction enzymes, which generated enzyme-specific profiles.

The *NAT2* profiles were analysed using a Mini-PROTEAN® II gel electrophoresis (BioRAd Laboratories, USA) and a 5% non-denaturing polyacrylamide gel. Gels were

run at 100 volts for 1 hour using 1 x TBE buffer. The DNA bands were then visualised and fixed by silver staining. The images were electronically scanned using Epson Scanner and supplied software (Siko Epsom, Japan)

4.5.5.2. Allele – Specific PCR Analysis

The *NAT2*5* is specifically identified by the 341 T>C nucleotide change. The confronting primer PCR technique (CP-PCR) was used to assess this polymorphism. Two primer sets were used, allele-specific confronting primer set (341T:^{1045} TTCTTCTGCAGGTGACCAT ^{1063} and 341C: ^{1081} ATGTAATTCCTGCCGTCAG ^{1063}) and an outer-primer set (A: ^{894} CACATTGTAAGAAGAAACCGG ^{914} and B: ^{1368} AAGATGTTGGAGACGTCTGC ^{1349}). A total volume of 25 µl PCR reaction consisted out of 20 ng genomic DNA, 0.25 mM dNTP mixture (dATP, dGTP, dTTP and dCTP), 2.5 µl 10 x PCR buffer (200 mM Tris, pH 8.4; 500 nM KCl) (Gibco BRL Life Technologies, USA), 2.5 mM $MgCl_2$, 0.25 µM of the four primers, and 0.25 µl *Taq* DNA Polymerase 1 (Gibco BRL Life Technologies, USA). The amplification was performed in the Gene Amp PCR System 9700 (Applied Biosystem, USA). The temperature cycling conditions were as follow: initial denaturation at 94ºC for 5 minute; 35 cycles of alternating denaturation at 94ºC, for 35 seconds; annealing at 58ºC for 45 seconds, polymerisation at 72ºC for 60 seconds, extended polymerisation at 72ºC for 10 minutes and finally a cool down to 4ºC for 60 minutes. Two micro litres of PCR product were assessed by gel electrophoresis using 2% agarose gel (BMA, USA), and 0.5 x TBE running buffer. The gels were run at 220 volts for 15 minutes, using Omeg gel apparatus and the RFLP bands were visual as previously described in the 'Agarose Gel Electrophoresis' section.

4.5.6. Genetic categorising

The genetic results were then used to categorise the subjects as either slow, fast or intermediate (one slow allele and one fast) acetylators for the *NAT1* and *NAT2* by using the available literature information.

Louisville medical school[h] (http://www.louisville.edu/medschool/pharmacology/) summarised the known genetic variants of *NAT1* and *NAT2*, and these were used as

a guideline. According to the summary of the Louisville medical school, the functional type of many of the *NAT1* variations is in fact unknown.
The genetic results were documented and the influence of *NAT1* and *NAT2* on the disposition of PAS was inspected by graphing the time-concentration profiles of each genotype against other genotypes combined. An interaction plot was generated to evaluate whether *NAT1* and *NAT2* have any interaction (described in '5.4.5 Effect of *NAT1* and *NAT2* polymorphisms')

4.6. Data analysis

Since the aim of the study was to compare the once and twice-daily regimens and investigate the possible co-factors involved in the different pharmacokinetic profiles of PAS, both statistical and pharmacometric analysis was performed.

4.6.1. Statistical analysis

In study 1 the body weight, BMI and pharmacokinetic results of occasion 1 and occasion 2 were statistically compared. In the comparison of the two occasions, only the data of the subjects who had completed both occasions were used.

In study 2, the body weight, BMI, pharmacokinetic results and tolerability (VAS scores) of once- and twice-daily regimens were compared by using cross-over statistical analysis. In this crossover comparison, only the data of the subjects who had completed both regimens were used. In addition, the pharmacokinetic profiles of the HIV positive and negative subjects of study 2 were compared.

The data of both studies were documented in a dataset using Microsoft Excel 2010. The independent variables were age, gender, race, height, body weight, BMI, VAS scores, concomitant medications and the results of the pharmacokinetic analysis. The variables were summarised using standard summary statistics (descriptive statistics). The summary statistic results were presented in tables and graphs using Microsoft Excel 2010 and Graph Pad Prism version 6.00 (GramphPad software, Inc. San Diego, California).

Statistical analysis was performed using the statistical programs SAS 9.1.3 and STATISTICA version 10. The comparison between groups (prescribed ART vs. not on ART) was treated as an interindividual (between-subject) effect and the difference between the regimens and occasions as intraindividual (within-subject) effect. Normality of the subject characteristics, pharmacokinetic parameters and the VAS scores were analysed using the Sharpiro-Wilks normality tests. The significant difference was then established by mixed analysis of variance model (ANOVA), Wilcoxon rank sum test or Wilcoxon match pair test. A statistically significant difference was defined as $p< 0.05$.

4.6.2. Population Pharmacokinetic analysis

4.6.2.1. Datasets used in the model development

The plasma concentration-time data of GSR-PAS administered as once- and twice-daily came from rich sampling design. Available data were used for the population pharmacokinetic analysis. The aim of the study was to determine the pharmacokinetic profile for GSR-PAS given once-daily versus twice-daily.

4.6.2.2. Population Pharmacokinetic Model

Population pharmacokinetic models were built using a non-linear mixed-effects modelling approach. The first-order conditional maximum likelihood estimation in the NONMEM program (double precision, Version 7.2, ICON Development Solutions, Elliott City, MD) and NM-TRAN pre-processor were used. Models were run using the G-Fortran Compiler (GNU Compiler Collection) on a personal computer under the Microsoft Windows 7.0 operating system (Dell Optiplex 755). PerlSpeaksNONMEM 3.5.5 (http://psn.sourceforge.net/) running activePerl 5.10.1 (ActiveState Software Inc., Vancouver, BC, Canada) were used to manage NONMEM batch files. The subroutines within NONMEM were linear mammillary models (ADVAN2 used with TRANS2 in the PREDPP library) to investigate a one-compartment, absorption model. The one-compartment open model with first order elimination with lag time provided the best fit to the dataset. The structural pharmacokinetic model for the two-compartment model consists of the following parameters: absorption rate constant (K_a), oral clearance (CL), volume of the central compartment (V), and lag-time (T_{lag}). The relative bioavailability parameter F assumed the value of 1. All pharmacokinetic

parameters were assumed to be log-normally distributed, and exponential interindividual variability terms were included in the pharmacokinetic parameters in the model. Interoccasional variability (IOV) was introduced to the model using the general model for IOV (Equation 1).

$$P_{ij} = \tilde{P} \times \exp(\eta_i + \kappa_{ij}) \qquad \text{Equation 1}$$

where:

ηi and κij to describe interindividual and interoccasion/ intraindividual (within an individual) variability are assumed to be independently, normally distributed parameters both with mean zero and variances ω_p^2 and π_p^2, respectively.

The allometric scaling based on body weight (BW) was applied to the PK parameters CL and V, as described by Equation 2

$$P_i = \tilde{P} \times \left(\frac{BW}{70\ kg}\right)^{\theta_1}$$

Equation 2

where:

$\tilde{P}$ is the parameter value for an individual body weight normalized to the ideal median body weight of the population of 70 kg and θ_1 is the allometric scaling exponent. For V, the allometric scaling parameter θ_1 was assumed to be 1.

Once the base model was established, covariate screening was carried using stepwise covariate model in PerlSpeaksNONMEM. The continuous covariate tested includes the demographic variable age. The categorical covariates included HIV status, gender and race

4.6.2.3. Covariate Analysis

Covariate screening was accomplished using a stepwise covariate model (SCM) in PerlSpeaksNONMEM (PsN). The hypothesis testing was to discriminate among alternative hierarchical structural models based on the p-values for the forward inclusion and backward elimination at 0.05 and 0.01, respectively. The linear and power relationships were explored for continuous covariates and exponential relationship for categorical bivariate. The resulting model that included all significant covariates was considered the “final” population pharmacokinetic model.

The concomitant medications were categorized according to their effect on cytochrome P450 (major drug metabolic group of enzymes). The categories were as follows: CYP1A2 substrate, inhibitor; CYP3A substrate, inhibitor, inducer; CYP2B6 substrate; CYP2C9 substrate, inhibitor; CYP2C19 substrate, inhibitor, inducer; CYP2D6 substrate, inhibitor; CYP2E1 inducer; CYP2C8 inducer; general inducer and inhibitor. Dummy variables consisting of 0 and 1 were introduced to each category for absence or presence of drug belonging to these categories, respectively. The influence of concomitant medications was evaluated using the SCM algorithm with the same forward inclusion and backward elimination probability values. Only exponential relationship was explored since these were categorical bivariates.

4.6.2.4. Model Validation

A degenerate visual predictive check was performed by the simulation of the parameter estimates of the final model to generate 1000 individual profiles. The median and 95% prediction intervals for the concentration at each time point were plotted and compared to the original data using R (v. 2.14.0).

The accuracy and robustness of the final model were evaluated using a non-parametric bootstrap procedure. The algorithm involves repeated random sampling of subjects available in the study, with replacement of the original dataset in each subsequent sampling to produce another dataset of the same size as the original, but with a different list of subjects. The re-sampling was repeated 500 times. The final population pharmacokinetic model was fitted to each of the bootstrap datasets and a set of model parameters were determined for each run. The median and 95% confidence intervals were computed and compared to the values from the original NONMEM analysis. The procedure was performed using PsN.

Chapter Five:
Results

5.1. Study 1: Clinical Results

5.1.1. Study Subjects

The data of forty one subjects (n=41) with DR-TB were documented. Data of forty subjects (n=40) were available at the first occasion and thirty nine subjects' (n=39) data were available at the second occasion. Thirty eight subjects (n=38) participated at both occasions and therefore their data were used in the comparison between the two occasions. The data of all the subjects (n=41) were used in the modelling and simulation analysis described in the population pharmacokinetic section. The demographic characteristics of all subjects are presented in Table 5.

Twenty subjects (49%) were female. Four females (20%) had XDR-TB and ten (50%) were HIV positive. The mean age of the females was 31 years (median: 28 years). The mean height was 163.9 cm (median: 164.0 cm) and the mean weight was 50.6 kg (median: 46.7 kg).

Twenty-one subjects (51%) were male. Two males (10%) had XDR-TB and two (10%) were HIV positive. The mean age of the males was 36 years (median: 33 years). The mean height was 170.8 cm (median: 169.5 cm) and the mean weight was 59.6 kg (median: 59.5 kg).

Table 5 Demographic characteristics of the subjects in study 1

Parameter	All the subjects (n= 41)
Gender (n, %)	
Female	20 (49%)
Male	21 (51%)
Race (n, %)	
Black	12 (29%)
Coloured	29 (71%)
Age (years)	
Mean	34
SD	11
CV%	34
Range	18-62
Median	31
TB Diagnosis	
MDR-TB	35 (85%)
XDR-TB	6 (15%)
HIV status	
Positive	12 (29%)
Negative	29 (71%)
Height (cm)	
Mean	168.3
SD	7.5
CV%	4
Range	149.0-183.0
Median	168.0
Weight (kg)	
Mean	55.6
SD	12.0
CV%	22
Range	33.0-84.0
Median	56.0

SD standard deviation; **CV** coefficient of variation

5.1.2. Concomitant medication

Six drugs of the standard XDR-TB regimen, including PAS were prescribed to the subjects in study 1. The anti-tuberculosis drugs and other antibiotics used are presented in Figure 8.

The first-line drugs prescribed were ethambutol (93%), pyrazinamide (93%) and INH (59%). Two subjects (5%) received the injectable, kanamycin and thirty one subjects (76%) received an alternative injectable, capreomycin. The fluoroquinolones prescribed were ofloxacin (59%) and moxifloxacin (2%); and the second-line oral bacteriostatic agents were terizidone (98%) and ethionamide (85%). Additional antimicrobial agents were clarithromycin (7%) and dapsone (10%).

All HIV positive subjects were on antiretroviral treatment. Figure 9 shows the concomitant antiretroviral drugs used by the HIV positive subjects. All HIV positive subjects were on lamivudine treatment. Eleven subjects (92%) received both efavirenz and stavudine. One subject (8%) was prescribed zidovudine (AZT), and one subject (8%) was prescribed lopinavir/ritonavir and lamivudine

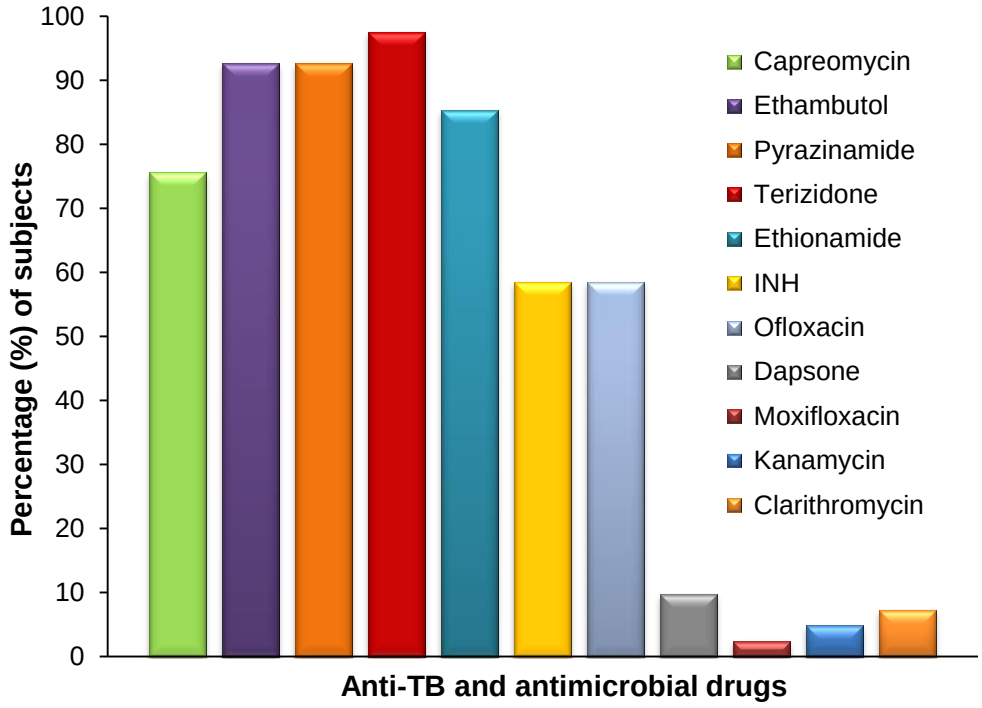

Figure 8 Anti-tuberculosis and antimicrobial drugs co-administered with GSR-PAS in study 1

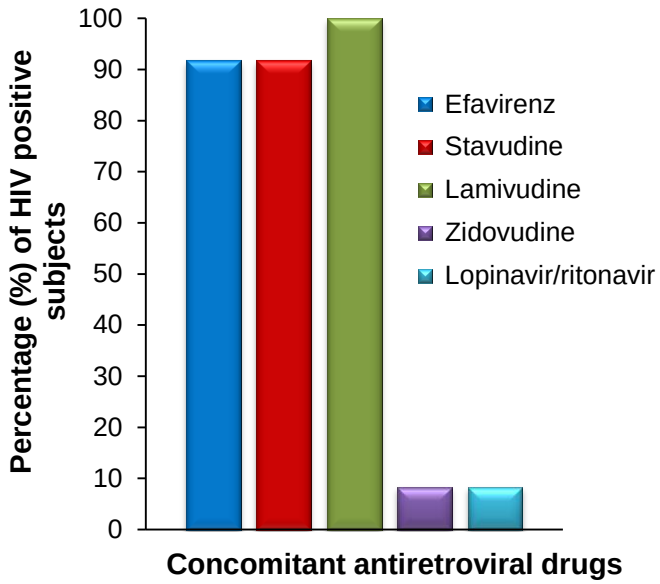

Figure 9 Concomitant antiretroviral drugs administered to the HIV positive subjects (n=12) in study 1

Sixteen subjects (39%) were prescribed pyridoxine (vitamin B6) and two (5%) subjects' vitamin B complex as a supplement. Eight subjects (20%) received antidepressants: amitriptyline (20%), fluoxetine (2%), and fluphenazine (2%). One subject (2%) was prescribed orphenadrine, and insulin and metformin for his type 2 diabetes. One subjects (2%) received eltroxin for hypothyroidism and two subjects (5%) ranitidine for peptic ulcer or heart burn. Figure 10 summarises the concomitant drugs used by the subjects in study 1.

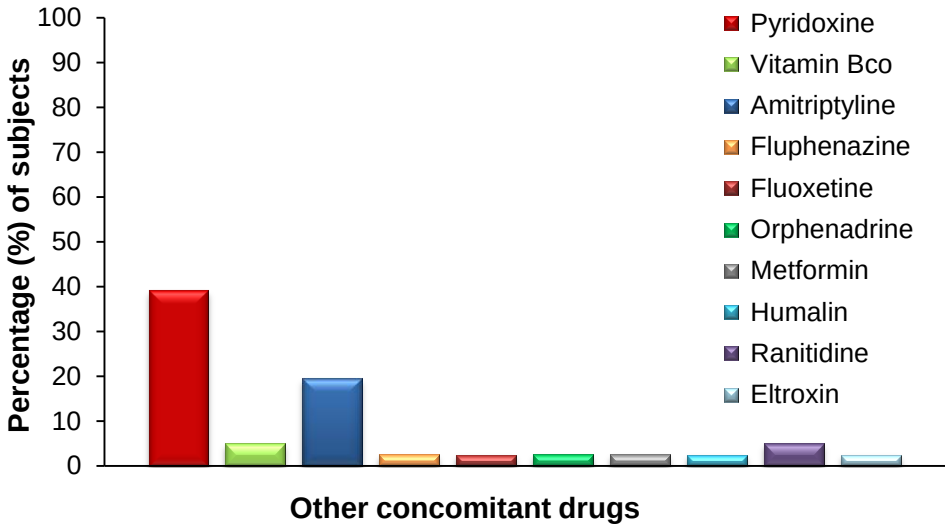

Figure 10 Other drugs co-administered with GSR-PAS in study 1

5.1.3. Comparison between first and second occasion

Only the thirty eight subjects that participated in both PK blood sampling occasions were used in the comparison of the two occasions. The demographic characteristics of the group of subjects are summarised in Table 6 and the weight in Table 7. Eighteen females (47%) and 20 (53%) males participated in both occasions.

Body weight and height measured on the PK sampling days were used for calculation of the BMI. Categories of BMI are as follows: underweight BMI<18.5 kg/m^2; normal weight 18.5-24.9 kg/m^2; overweight 25.0-29.9 kg/m^2 and obesity >30 kg/m^2. Six of the thirty eight subjects (16%) were underweight; thirty subjects (79%) had a normal weight; and one subject (3%) was obese.

Seven subjects' heights were not available; therefore the BMI of these subjects were not calculated. Mean body weight at the first occasion was 56.5 kg and 57.4 kg at the second occasion. Mean BMI was 20.5 kg/m^2 and 20.8 kg/m^2 at the first and second occasion, respectively.

Table 6 Demographic characteristics of subjects included in both occasions

Parameter	Both occasions (n=38)
Gender (n, %)	
Female	18 (47%)
Male	20 (53%)
Race (n, %)	
Black	12 (32%)
Coloured	26 (68%)
Age (years)	
Mean	34
SD	12
CV%	35
Range	18-62
Median	32
TB Diagnosis	
MDR	33 (87%)
XDR	5 (13%)
HIV status	
Positive	11 (29%)
Negative	27 (71%)
Height (cm)	
Mean	168.8
SD	7.4
CV%	4
Range	149.0-183.0
Median	169.0
Missing values (n, %)	7 (18%)

SD standard deviation; **CV** coefficient of variation

The weight and BMI were analysed for significant difference (Table 7). Body weight and BMI were considered as not normally distributed as verified by the Shapiro-Wilks normality test, therefore the Wilcoxon matched pairs test was used to assess whether there were any significant differences between the two occasions (level of significance set at $p<0.05$).

Body weight ($p=0.011$) and BMI ($p=0.007$) were significantly higher at the second occasion; however the differences were only small and not clinically relevant.

Table 7 Comparison between body weight and BMI of the same subjects recorded at the first and second occasion in study 1

Parameter	**Occasion 1 (n=38)**	**Occasion 2 (n=38)**	**p-value***
Body weight (kg)			
Mean	56.5	57.4	0.011
SD	11.9	12.1	
CV%	21	21	
Range	33.0-84.0	34.7-83.0	
Median	57	59.0	
Missing values (n, %)	0 (0%)	1 (3%)	
Body Mass Index (BMI=kg/m^2)			
Mean	20.5	20.8	0.007
SD	3.2	3.3	
CV%	16	16	
Range	14.4-30.9	14.0-30.5	
Median	20.6	21.2	
Missing values (n, %)	7 (18%)	7 (18%)	

* Indicates significance ($p < 0.05$); **SD** standard deviation; **CV** coefficient of variation

5.2. Study 2: Clinical Results

5.2.1. Study Subjects

Thirty two subjects (n=32) were eligible for PK blood sampling in study 2. Twenty nine subjects (n=29) followed the once- and twice-daily regimens for two separate weeks (crossover) and therefore their data were used in the comparison between the two regimens (this group completed both regimens). The data of all the subjects (n=32) were used in the modelling and simulation analysis described in the population pharmacokinetic section. The demographic characteristics of the study subjects are presented in Table 8.

Fourteen subjects (44%) were female. Two females (14%) had XDR-TB and three (21%) were HIV positive. The mean age of the females was 37 years (median: 36 years) and mean height was 158.3 cm (median: 158.5 cm).

Eighteen subjects (56%) were male. Six males (33%) had XDR-TB and six (33%) were HIV positive. The mean age of the males was 35 years (median: 34 years) and mean height was 171.0 cm (median: 170.0 cm).

Serum creatinine, estimated glomerular filtration rate (eGFR) as determined by the Cockcroft & Gault formula[99] and alanine aminotransferase (ALT) are presented in Table 9. Not all of the above mentioned clinical laboratory test results were available for each subject; the number of missing values is recorded in the table.

Table 8 Demographic characteristics of subjects in study 2

Parameter	All the subjects (n=32)
Gender (n, %)	
Female	14 (44%)
Male	18 (56%)
Race (n, %)	
Black	5 (16%)
Coloured	27 (84%)
Age (years)	
Mean	36
SD	11
CV%	31
Range	18-64
Median	34
HIV Status (n, %)	
Positive	9 (28%)
Negative	23 (72%)
TB Diagnosis (n, %)	
MDR	24 (75%)
XDR	8 (25%)
Height (cm)	
Mean	165.0
SD	9.0
CV%	5
Range	150.0-183.0
Median	167.0
Weight (kg)	
Mean	56.1
SD	10.0
CV%	18
Range	42.0-85.5
Median	54.5

SD standard deviation; **CV** coefficient of variation

Table 9 Clinical laboratory results in study 2

Parameter	All the subjects (n=32)
Serum creatinine (µmol/l)	
Mean	81
SD	27
CV%	33
Range	44–152
Median	73.0
Missing values (n, %)	0 (0%)
eGFR (ml/min)	
Mean	92
SD	37
CV%	41
Range	37–206
Median	88
Missing values (n, %)	0 (0%)
ALT (U/L)	
Mean	17
SD	12
CV%	71
Range	5–57
Median	16
Missing values (n, %)	16 (47%)

SD standard deviation; **CV** coefficient of variation

5.2.2. Concurrent diseases

Three subjects (9%) were infected with *Treponema pallidum* (syphilis); two (67%) of these subjects were HIV positive. Four subjects (13%) suffered from diabetes mellitus (DM) and two subjects (6%) from hypothyroidism. Ten subjects (31%) had depression and two (6%) suffered from anxiety. Two subjects (6%) were diagnosed with a psychotic disorder, but were stable.

5.2.3. Concomitant medication

Seven drugs of the standard XDR-TB regimen, including PAS were prescribed to the subjects in study 2. The anti-tuberculosis drugs and other antibiotics used are presented in Figure 11.

The anti-TB drugs were capreomycin (88%), moxifloxacin (97%), ethionamide (88%), terizidone (97%), pyrazinamide (84%) ethambutol (84%), INH (47%), clofazimine (38%), dapsone (6%) and kanamycin (6%). Additional antimicrobial agents were clarithromycin (3%) and co-trimoxazole (6%).

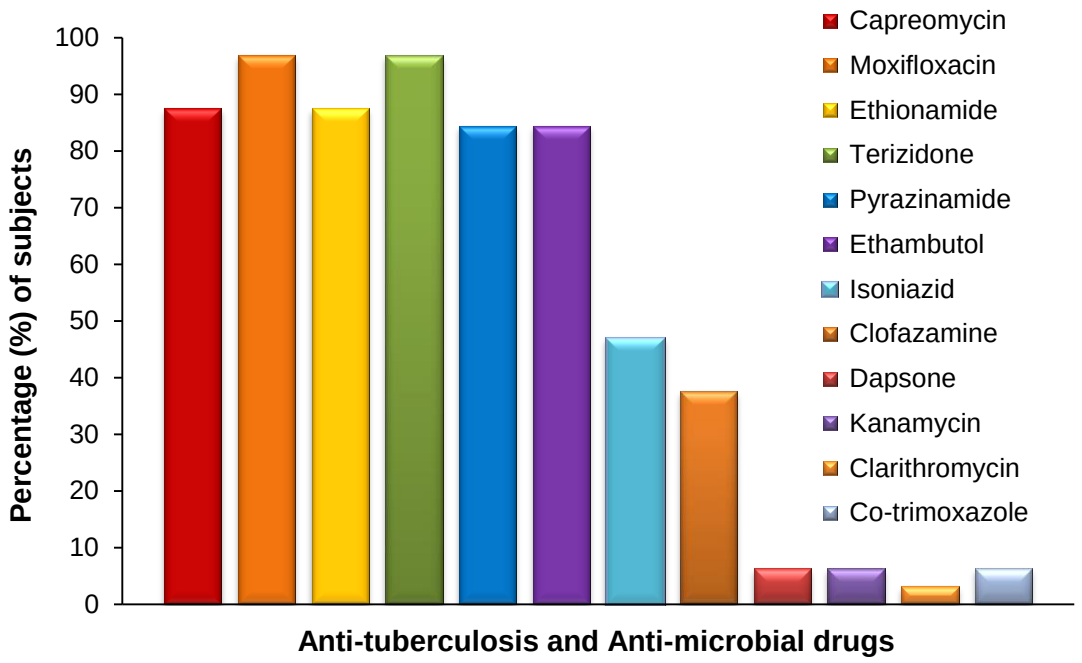

Figure 11 Anti-tuberculosis and antimicrobial drugs co-administered with GSR-PAS in study 2

All HIV positive subjects were on antiretroviral treatment as shown in Figure 12. Eight subjects were prescribed lamivudine and efavirenz (89%). Seven of these subjects were also on stavudine (78%) and one was on zidovudine (11%). One subject was prescribed a lopinavir/ritonavir and lamivudine/zidovudine regimen.

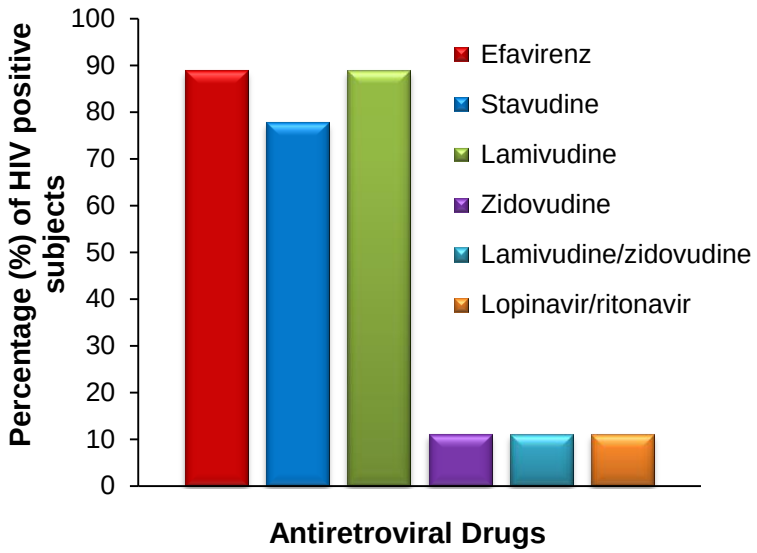

Figure 12 Concomitant antiretroviral drugs administered to the HIV positive subjects (n=9) in study 2

Four diabetic subjects (12.5%) were treated with metformin (50%), glibenclamide (25%), and insulin (actraphane 25% and humulin 50%) (See Figure 13).

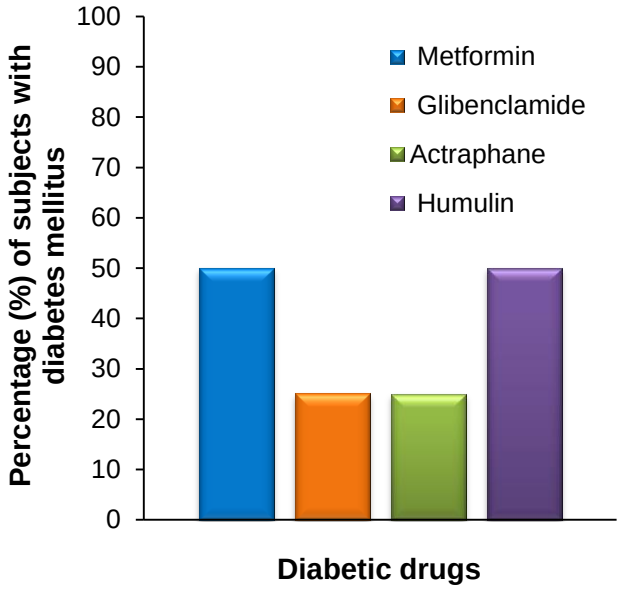

Figure 13 Concomitant diabetic drugs administered to the diabetic subjects (n=4) in study 2

Ten subjects (31%) were treated for depression with amitriptyline, and one subject was also prescribed fluoxetine. Two subjects (6.3%) suffered from anxiety and were prescribed lorazepam and diazepam, respectively. Two subjects (6.3%) had a psychotic disorder and were treated with haloperidol and chlorpromazine, respectively. These drugs are outlined in Figure 14.

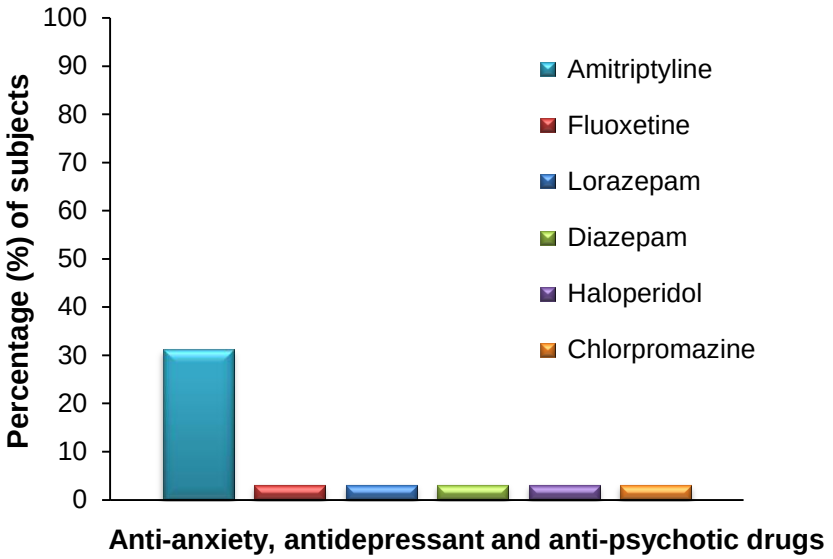

Figure 14 Anti-anxiety, anti-depressant and/or antipsychotic drugs co-administered with GSR-PAS in study 2

Other concomitant drugs and dietary supplements are shown in Figure 15. Pyridoxine (97%), vitamin B complex (47%), vitamin C (16%), potassium (6%), folic acid (3%) and magnesium (3%) were prescribed as dietary supplements. Other medications were eltroxin (6%), maxulon (31%), ranitidine (13%), orphenadrine (9%), codeine phosphate (9%), aspirin (3%), amlodipine (3%) and $FeSO_4$ (3%).

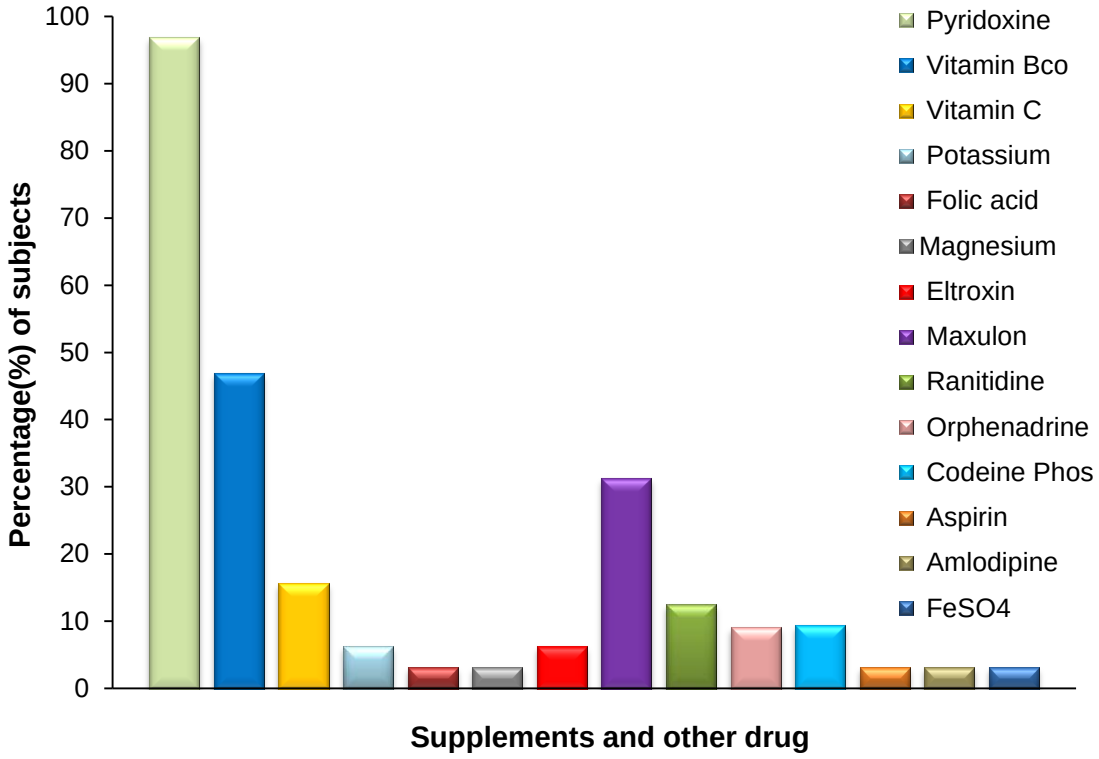

Figure 15 Other drugs and dietary supplements co-administered with GSR-PAS in study 2

5.2.4. Demographic data of subjects included in both cross-over periods

Twenty nine (n=29) subjects were eligible for the cross-over comparison. On each PK sampling day the weight and height were measured with standard measuring instruments, and the BMI was calculated accordingly. The demographic characteristics and the weight are summarised in Table 10 and 11, respectively. Twelve subjects (41%) were female and 17 (59%) were male. Twenty four (83%) subjects were Coloured and five subjects (17%) were Black. The mean age was 36 years (median 34 years). Nine subjects (31%) were HIV positive and seven subjects (24%) had XDR-TB.

Table 10 Demographic characteristics of subjects included in both cross-over periods

Parameter	Cross-over (n = 29)
Gender no. (%)	
Female	12 (41%)
Male	17 (59%)
Race no. (%)	
Black	5 (17%)
Coloured	24 (83%)
Age (years)	
Mean	36
SD	11
CV%	31
Range	18-64
Median	34
HIV Status	
Positive (n, %)	9 (31%)
Negative (n, %)	20 (69%)
TB Diagnosis	
MDR	22 (76%)
XDR	7 (24%)
Height (cm)	
Mean	165.9
SD	8.3
CV%	5
Range	150.0-183.0
Median	167.0

SD standard deviation; **CV** coefficient of variation

Table 11 Comparison between body weight and BMI recorded at the start of both treatment regimens in study 2

Parameter	Dosing Regimens of GSR- PAS		
	Twice-daily (n=29)	**Once-daily (n=29)**	**p-value***
Body weight (kg)			
Mean	55.5	55.1	0.065
SD	8.5	8.7	
CV%	15	16	
Range	42.0-73.0	40.0-71.5	
Median	56.0	56.0	
Body Mass Index (BMI= kg/m^2)			
Mean	20.2	20.0	0.060
SD	2.5	2.7	
CV%	12	13	
Range	16.0-25.6	15.1-25.2	
Median	19.9	19.8	

* Indicates significance ($p < 0.05$); **SD** standard deviation; **CV** coefficient of variation

The mean differences for body weight and BMI between the once-and twice-daily regimens was 0.4 kg and 0.2 kg/m^2 which were not significantly different. Two subjects who started with the twice-daily regimen lost a remarkable amount of weight in a week (6.5 kg and 7.0 kg).

5.3. Pharmacokinetic profiles and non-compartmental analysis

Most of the blood samples in both studies were collected at the scheduled times. A time window of 10 minutes was allowed for each sample. In a few cases, it was not possible to obtain some of the samples at the scheduled times, due to intervening clinical events. The dosing and blood sampling times were accurately recorded and documented for each subject.

5.3.1. Study 1: Pharmacokinetic data

A mean pharmacokinetic profile for PAS during administration of GSR-PAS at a dose of 4 g twice-daily was generated for each occasion to visually examine any differences between the two study days (occasions). Figure 16 depicts the pharmacokinetic profiles at the two occasions. Mean concentrations were higher at the first than at the second occasion.

The individual pharmacokinetic results are available in Appendix J. The summary statistics of the pharmacokinetic parameters obtained after administration of 4 g GSR-PAS twice-daily at the two different occasions are presented in Table 12. The Wilcoxon matched pairs test was used to assess whether the parameters were significantly different between the two occasions ($p<0.05$).

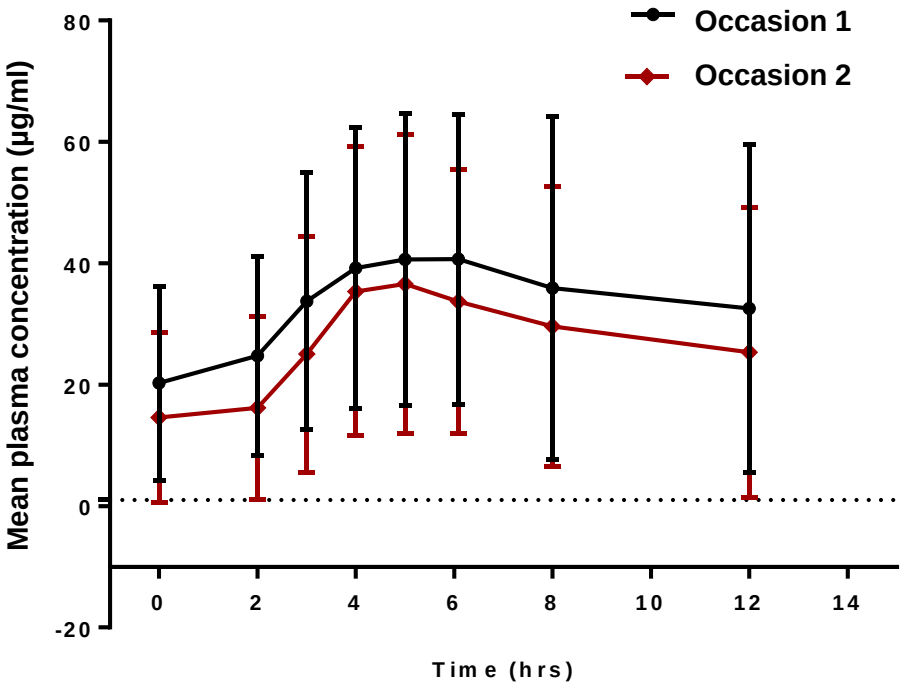

Figure 16 Mean (standard deviation) plasma PAS concentration in subjects receiving 4 g twice-daily GSR-PAS dose on occasion 1 and occasion 2

Table 12 Summary statistics for the pharmacokinetic parameters of GSR-PAS (4 g twice-daily, steady state conditions) at both occasions

PK Parameter	Occasion 1 (N=38)	Occasion 2 (N=38)	P-value*
C_{max} (µg/ml)			
Mean	51.97	45.20	0.141
SD	25.21	22.37	
CV%	49	49	
Range	13.35-119.00	11.25-119.00	
Median	44.63	40.20	
t_{max} (h)			
Mean	4.70	4.63	-
SD	2.20	2.16	
CV%	49	47	
Range	0.00-8.00	0.00-8.00	
Median	5.00	4.02	
C_{min} (µg/ml)			
Mean	14.82	10.29	0.078
SD	14.80	12.38	
CV%	100	120	
Range	0.23-57.80	0.21-57.80	
Median	8.77	7.61	
C_0 (µg/ml)			
Mean	20. 27	14.60	0.027
SD	16.00	13.94	
CV%	79	95	
Range	1.19-57.80	0.21-57.80	
Median	20.10	11.25	
t_{min} (h)			
Mean	3.37	2.95	-
SD	4.47	3.62	
CV%	133	123	
Range	0.00-12.00	0.00-12.00	
Median	1.50	2.00	

* Indicates significance ($p < 0.05$); **SD** standard deviation; **CV** coefficient of variation

Table 12 Summary statistics for the pharmacokinetic parameters of GSR-PAS (4 g twice-daily, steady state conditions) at both occasions (continued)

PK Parameter	Occasion 1 (N=38)	Occasion 2 (N=38)	P-value*
AUC_{last}			
Mean	305.48	242.41	0.028
SD	181.56	147.26	
CV%	59	61	
Range	56.38-852.06	42.09-726.80	
Median	289.65	205.20	
C_{last}			
Mean	31.73	25.12	0.180
SD	24.08	19.80	
CV%	76	79	
Range	1.80- 91.50	1.03-89.30	
Median	27.48	20.38	

* Indicates significance ($p < 0.05$); **SD** standard deviation; **CV** coefficient of variation

The mean maximum concentrations (C_{max}) at the first and second occasion were 51.97 µg/ml (median: 44.63 µg/ml) and 45.20 µg/ml (median: 40.20 µg/ml, respectively. The mean minimum concentration was 14.82 µg/ml (median: 8.77 µg/ml) at the first occasion and 10.29 µg/ml (median 7.61 µg/ml) at the second occasion.

In two subjects the minimum concentrations were less than the MIC of 1 µg/ml at occasion 1 and were detected at 3 and 4 hours, respectively. In contrast to occasion 1, minimum concentrations were below the MIC in eight subjects at occasion 2. Minimum concentrations were recorded at time zero in seven of the eight subjects.

The mean concentration at time zero ($p> 0.027$) and the area under the curve from zero to the last concentration, was significantly ($p> 0.028$) higher at the first than at the second occasion. There was no significant difference between the first and second occasion for the maximum and minimum concentrations.

5.3.2. Study 2: Pharmacokinetic data

The means of both pharmacokinetic profiles (4 g twice-daily and 8 g once-daily at steady state conditions) are presented in Figure 17. The twenty-four hour level of the profiles is connected with dashed lines, since no blood samples were obtained during the night. As expected, the 8 g once-daily regimen produced a much higher peak concentration than the 4 g twice-daily regimen. The peak concentration occurred later in the once-daily regimen.

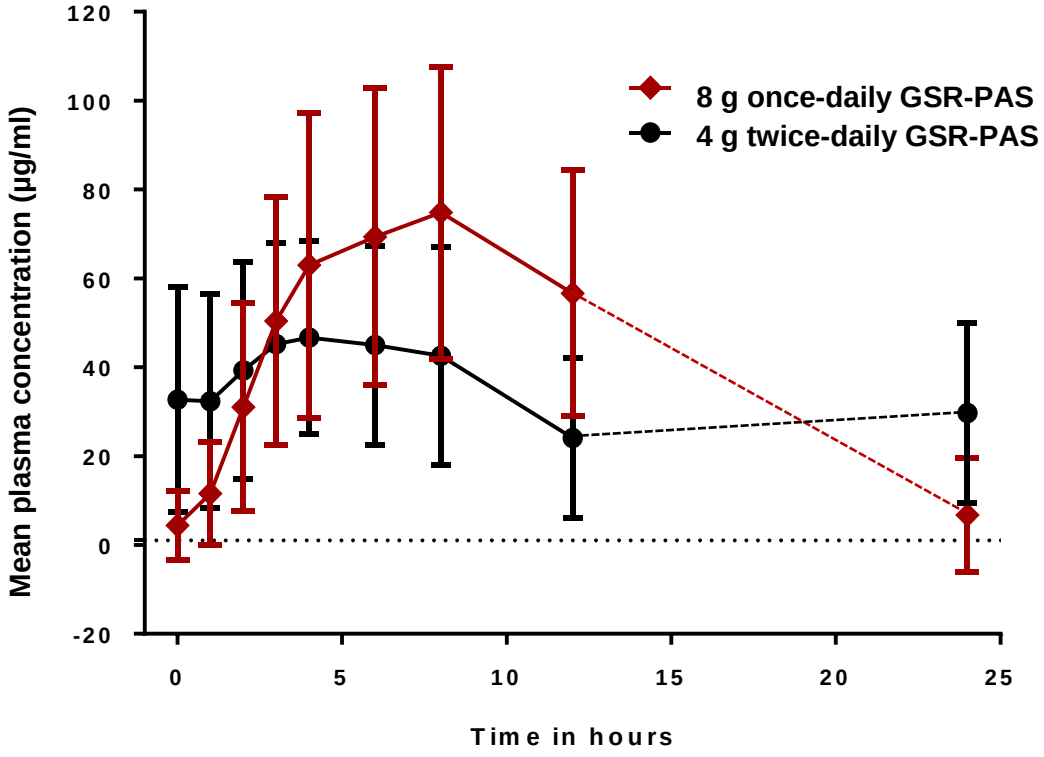

Figure 17 Mean (standard deviation) plasma PAS concentration in subjects receiving 8 g once-daily or 4 g twice-daily GSR-PAS dose

The individual parameters are available in Appendix K. Table 13 summarises the statistics for the pharmacokinetic parameters of both dosing regimens.

Once-daily regimen

The C_{max} and C_{min} were 86.88 µg/ml (median: 80.00 µg/ml) and 3.42 µg/ml (median: 0.93 µg/ml), respectively. The mean area under the curve to twelve hours (AUC_{12}) was 665.55 µg.h/ml (median: 651.95 µg.h/ml). The estimated (oral) clearance and volume of distribution were 8.85 L/h (median: 7.53 L/h) and 35.32 L (median: 29.70 L), respectively.

The plasma concentrations of all subjects were maintained above the MIC of PAS (1 µg/ml) from dosing to the twelve hour sample. The mean concentration at twelve (C_{12}) hours was 56.63 µg/ml (median: 59.30 µg/ml).

PAS concentrations were detected in the 24 hour samples of all subjects, except one. PAS plasma concentrations at 24 hour were above the MIC (range: 1.28-53.6 µg/ml) in eighteen subjects (62.1%). The mean concentrations at time zero (C_0) and at 24 hours (C_{24}) (both pre-dose) were 4.39 µg/ml (median: 0.98 µg/ml) and 6.71 µg/ml (median: 1.36 µg/ml). The mean half-life of PAS was estimated to be 2.91 hours (median: 2.56 hours).

Twice-daily regimen

The mean C_{max} and C_{min} were 58.19 µg/ml (median: 60.70 µg/ml) and 19.66 µg/ml (median: 18.90 µg/ml), respectively. The mean AUC_{12} was 470.75 µg.h/ml (median: 428.27 µg.h/ml). The estimated (oral) clearance and volume of distribution were 10.96 L/h (median: 9.34 L/h) and 45.20 L (median: 37.71 L), respectively.

PAS concentrations were maintained above the MIC (1 µg/ml) throughout the twice-daily dosing interval. The mean C_0 and C_{12} (both pre-dose) were 32.73 µg/ml (median: 27.80 µg/ml) and 24.10 µg/ml (median: 21.40 µg/ml). The mean half-life of PAS was 6.80 hours (median: 3.85 hours)

Wilcoxon rank sum test was used to assess whether the parameters of the two regimens were significantly different ($p<0.05$). The mean C_{12}, C_{max}, AUC_{12} and the t_{max} were significantly higher in the once-daily regimen ($p<0.0001$, $p<0.0004$, $p< 0.009$ and 0.004, respectively). In contrast, the mean C_{min}, C_0 and C_{24} were significantly higher for the twice-daily regimen ($p<0.0001$ for all three mean concentrations). There was no significant difference for the t_{min}, CL_{ss}/F and t½.

Table 13 Summary statistics for the pharmacokinetic parameters of GSR-PAS after steady state administration in 4 g twice-daily versus 8 g once-daily regimens

PK Parameter	Dosing Regimens of GSR- PAS		P-value*
	4 g twice-daily (N=29)	8 g once-daily (N=29)	
C_{max} (µg/ml)			
Mean	58.19	86.88	0.0004
SD	24.58	29.12	
CV%	42	34	
Range	9.92-112.00	20.70-135.00	
Median	60.70	80.00	
t_{max} (h)			
Mean	5.14	7.38	0.004
SD	3.52	2.97	
CV%	68	40	
Range	0.00-12.00	3.00-12.05	
Median	4.03	8.00	
C_{min} (µg/ml)			
Mean	19.66	3.42	<0.0001
SD	16.02	6.86	
CV%	82	201	
Range	0.80-63.20	0.00-28.60	
Median	18.90	0.93	
t_{min} (h)			
Mean	7.89	6.97	0.709
SD	5.00	10.78	
CV%	63	155	
Range	0.00-12.07	0.00-24.00	
Median	11.95	0.00	

* Indicates significance ($p < 0.05$); **SD** standard deviation; **CV** coefficient of variation

Table 13 Summary statistics for the pharmacokinetic parameters of GSR-PAS after steady state administration in 4 g twice-daily versus 8 g once-daily regimens (continued)

PK Parameter	Dosing Regimens of GSR- PAS		P-value*
	4 g twice-daily (N=29)	8 g once-daily (N=29)	
C_0 (µg/ml)			
Mean	32.73	4.39	<0.0001
SD	25.30	7.73	
CV%	77	176	
Range	1.46-105.00	0.00-28.60	
Median	27.80	0.98	
C_{12} (µg/ml)			
Mean	24.10	56.63	<0.0001
SD	18.02	27.67	
CV%	75	49	
Range	1.73-63.20	20.60-121.00	
Median	21.40	59.30	
C_{24} (µg/ml)			
Mean	29.71	6.71	<0.0001
SD	20.36	12.87	
CV%	69	192	
Range	2.36-81.90	0.03-53.60	
Median	22.40	1.36	
AUC_{12} (µg.h/ml)			
Mean	470.75	665.55	0.009
SD	232.47	268.81	
CV%	49	40	
Range	119.30-1022.49	161.09-1055.37	
Median	428.27	651.95	

* Indicates significance ($p < 0.05$); **SD** standard deviation; **CV** coefficient of variation

Table 13 Summary statistics for the pharmacokinetic parameters of GSR-PAS after steady state administration in 4 g twice-daily versus 8 g once-daily regimens (continued)

PK Parameter	Dosing Regimens of GSR- PAS		
	4 g twice-daily (N=29)	8 g once-daily (N=29)	P-value*
t½ (h)			
Mean	6.80	2.91	0.058
SD	10.43	1.34	
CV%	153*	46	
Range	1.36-47.06	1.42-6.77	
Median	3.85	2.56	
CL_{ss}/F (L/h)			
Mean	10.96	8.85	0.120
SD	6.65	3.95	
CV%	61	45	
Range	3.91-33.53	4.23-19.39	
Median	9.34	7.53	
V_z/F (L)			
Mean	45.20	35.32	0.026
SD	25.78	21.68	
CV%	57	61	
Range	19.47-119.71	11.61-112.72	
Median	37.71	29.70	

* Indicates significance ($p < 0.05$); **SD** standard deviation; **CV** coefficient of variation

5.3.3. Study 2: Pharmacokinetic data of subjects prescribed ART

The nine HIV positive subjects were prescribed ART. The mean age of the HIV positive subjects was 35 years (median: 34 years) and the mean weight was 54.3 kg (median: 50.5 kg). The mean age and weight of the HIV negative subjects were 35.9 years (median: 33.5 years) and 56.2 kg (median: 57.5 kg), respectively.

Mean pharmacokinetic profiles were generated for both the HIV positive and negative subjects receiving the different regimens. Figure 18 depicts mean pharmacokinetic

profiles of the HIV positive and negative subjects' on the twice-daily regimen and Figure 19 shows the once-daily regimen.

The summary statistics of the pharmacokinetic parameters for the HIV positive and HIV negative subjects (4 g twice-daily and 8 g once-daily GSR-PAS doses at steady state conditions) are presented in Table 14. Since none of these parameters were normally distributed, the Wilcoxon rank sum test was used to assess whether the PK parameters for HIV positive and HIV negative subjects ($p<0.05$) were significantly different.

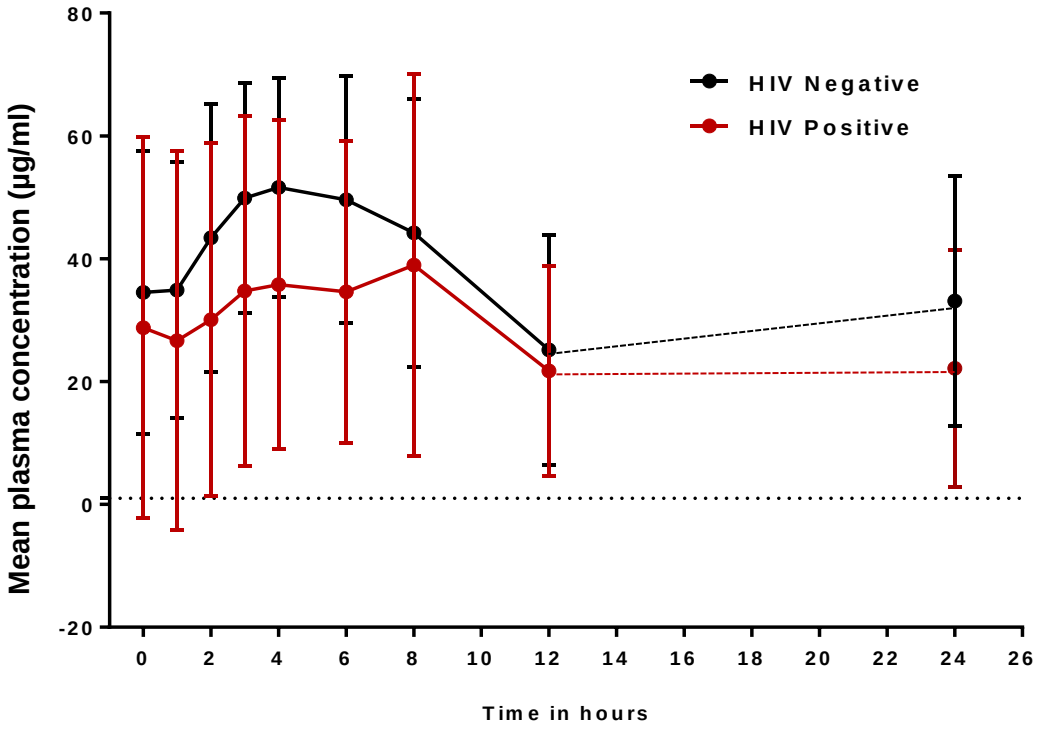

Figure 18 Mean (standard deviation) plasma PAS concentration in HIV positive and negative subjects receiving 4 g twice-daily GSR-PAS dose

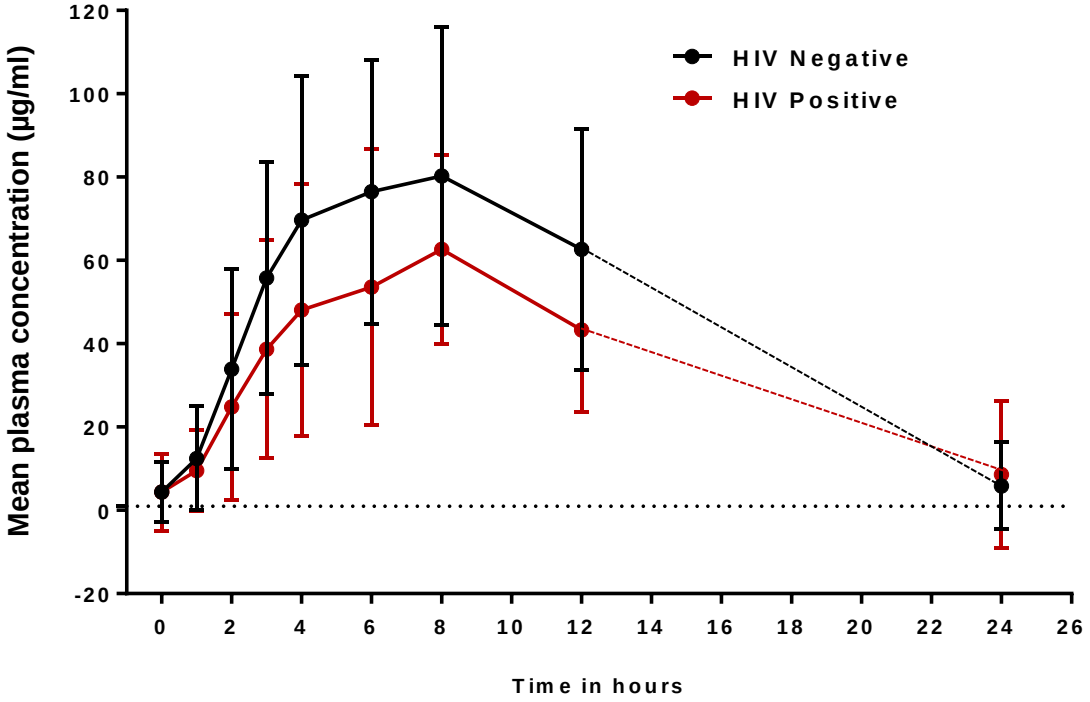

Figure 19 Mean (standard deviation) plasma PAS concentration in HIV positive and negative subjects receiving 8 g once-daily GSR-PAS dose

In both regimens PAS concentrations were remarkably lower for the HIV positive subjects. For the once-daily regimen, the mean AUC_{12} of the HIV negative subjects (mean: 727.26 µg/ml) was significantly (p-value: 0.043) larger than the HIV positive subjects (mean: 528.42 µg/ml). The significant higher mean AUC_{12} was only seen with once-daily dosing and not with twice-daily dosing.

The mean C_{max} of the HIV positive and negative subjects for the once-daily regimen was 72.56 µg/ml (median: 73.40 µg/ml) and 93.33 µg/ml (median: 98.40 µg/ml); and for the twice-daily regimen it was 49.64 µg/ml (median: 39.70 µg/ml) and 62.04 µg/ml (median: 61.80 µg/ml), respectively. There was a large variation of PAS plasma concentration at time zero and twenty four hours for both regimens. Only three of the nine (33.3%) HIV positive subjects' PAS concentrations were maintained above the MIC throughout the once-daily dosing interval, while all but one subject's minimum concentration (8 of 9, 89%) were above the MIC during the twice-daily dosing interval. Nine of the twenty (45%) HIV negative subjects' minimum concentrations were above the MIC for the once-daily dosing interval, while all minimum concentrations were above the MIC during the twice-daily dosing interval.

Table 14 Summary statistics for the pharmacokinetic parameters of GSR-PAS in HIV positive and negative subjects (after steady state administration in 4 g twice-daily versus 8 g once-daily regimens)

	Dosing Regimens of GSR- PAS					
	4 g twice-daily			**8 g once-daily**		
PK Parameter	**HIV Pos (n=9)**	**HIV Neg (n=20)**	**P-value***	**HIV Pos (n=9)**	**HIV Neg (n=20)**	**P-value***
C_{max} (µg/ml)						
Mean	49.64	62.04	0.144	72.56	93.33	0.081
SD	31.35	20.53		18.55	31.04	
CV%	64	33		26	33	
Range	9.92-105.00	27.60-112.00		47.80-111	20.70-135.00	
Median	39.70	61.80		73.40	98.40	
t_{max} (h)						
Mean	5.56	4.95	-	8.00	7.11	-
SD	4.51	3.09		2.65	3.13	
CV%	81	62		33	44	
Range	0.00-12.00	0.00-11.98		0.25-28.60	3.00-12.05	
Median	6.00	4.02		0.77	8.00	
t_{min} (h)						
Mean	7.32	8.14		5.79	7.50	-
SD	5.09	5.08		10.41	11.16	
CV%	69	62		180	149	
Range	0.00-12.00	0.00-12.07		0.00-24.00	0.00-24.00	
Median	7.97	11.95		0.00	0.00	
C_0 (µg/ml)						
Mean	28.75	34.52	0.258	4.37	4.40	0.777
SD	31.01	22.97		9.25	7.22	
CV%	108	67		211	164	
Range	1.46-105	3.79-100.00		0.33-28.60	0.00-21.30	
Median	19.80	32.00		0.77	1.15	

* Indicates significance ($p < 0.05$); **SD** standard deviation; **CV** coefficient of variation

Table 14 Summary statistics for the pharmacokinetic parameters of GSR-PAS in HIV positive and negative subjects (after steady state administration in 4 g twice-daily versus 8 g once-daily regimens) (continued)

	Dosing Regimens of GSR- PAS					
	4 g twice-daily			**8 g once-daily**		
PK Parameter	**HIV Pos (n=9)**	**HIV Ned (n=20)**	**P-value***	**HIV Pos (n=9)**	**HIV Neg (n=20)**	**P-value***
C_{min} (µg/ml)						
Mean	16.87	20.9	0.239	4.06	3.10	0.888
SD	18.73	15.0		9.25	5.80	
CV%	111	72		228	184	
Range	0.8-63.2	1.73-51.2		0.25-28.60	0.00-21.30	
Median	13.20	21.95		0.77	0.94	
C_{12} (µg/ml)						
Mean	21.73	25.17	0.850	43.28	62.65	0.099
SD	17.05	18.77		19.73	29.02	
CV%	78	75		46	46	
Range	8.75-63.2	1.73-62.80		20.90-7.60	20.6-121.00	
Median	15.40	21.95		44.10	62.10	
C_{24} (µg/ml)						
Mean	22.15	33.11	0.090	8.59	5.86	0.925
SD	19.25	20.38		17.69	10.48	
CV%	87	62		206	179	
Range	5.76-67.00	2.36-81.90		0.25-53.60	0.03-41.70	
Median	16.10	32.25		1.36	1.35	
AUC_{12} (µg.h/ml)						
Mean	410.35	500.96	0.205	528.42	727.25	0.043
SD	292.20	201.97		208.16	274.46	
CV%	71	40		39	38	
Range	119.30-1022.49	197.73-934.09		252.31-888.22	161.09-1055.37	
Median	327.74	500.06		576.05	804.76	

* Indicates significance ($p < 0.05$); **SD** standard deviation; **CV** coefficient of variation

Table 14 Summary statistics for the pharmacokinetic parameters of GSR-PAS in HIV positive and negative subjects (after steady state administration in 4 g twice-daily versus 8 g once-daily regimens) (continued)

	Dosing Regimens of GSR- PAS					
	4 g twice-daily			**8 g once-daily**		
PK Parameter	**HIV Pos (n=9)**	**HIV Neg (n=20)**	**P-value***	**HIV Pos (n=9)**	**HIV Neg (n=20)**	**P-value***
AUC_{24} (µg.h/ml)						
Mean	647.99	592.95	0.930	859.10	1140.77	0.140
SD	495.15	331.28		321.29	406.93	
CV%	76	56		37	36	
Range	256.34-1627.17	229.70-1370.86		412.52-1357.25	533.79-1892.72	
Median	492.66	541.35		889.57	1215.96	
t½ (h)						
Mean	13.14	3.87	0.161	2.62	3.03	0.764
SD	17.41	2.76		0.89	1.50	
CV%	133	71		34	50	
Range	3.04-47.06	1.36-11.54		1.85-4.38	1.42-6.77	
Median	4.43	3.51		2.56	2.55	
CL_{ss}/F (L/h)						
Mean	14.12	9.37	0.2047	10.90	8.04	0.140
SD	9.58	4.19		5.11	3.18	
CV%	68	45		47	40	
Range	3.91-33.53	4.28-20.23		5.89-19.36	4.23-14.99	
Median	12.20	8.00		9.00	6.58	
V_z/F (L)						
Mean	62.98	37.00	0.028	45.83	30.72	0.285
SD	30.94	19.16		32.05	14.28	
CV%	49	52		70	46	
Range	35.13-119.71	19.47-94.88		19.74-112.72	11.61-58.59	
Median	58.67	36.74		34.07	29.67	

* Indicates significance ($p < 0.05$); **SD** standard deviation; **CV** coefficient of variation

5.4. Genetic data

Genetic analysis was performed in sixty one subjects. *NAT1* variants were analysed in the first study, while both *NAT1* and *NAT2* were successfully determined in the second study. The individual genetic results of study 1 and 2 are presented in Appendix L and M.

Since various genotypes were identified in a low sample size for genetics, the results of the two studies were combined. Table 15 summarises the *NAT1* genotype distribution of all the subjects, including separation by their ethnic groups. Table 16 summarises the *NAT2* genotype distribution in study 2 and also includes the ethnic group separation.

Sixteen subjects (26%) have the *NAT 1*4/1*4* genotype, also known as the wild-type. The predominant alleles (haplotypes) were *NAT1*4* and *NAT1*10*. The other alleles identified, were *NAT1*4A*, *NAT1*27* and *NAT1*3*.

One subject had a variation at nucleotide position 1088 and was a homozygote for this variation. Since this variation was not defined/ described in the *NAT1* allele database, it was not classified. The functional type of *NAT1*10, NAT1*3* and *NAT1*4A* is classified as unknown (according to the summary of Louisville medical school.

Seven subjects (22%) of study 2 were identified to be wild-type or fast acetylators of *NAT2*, while eleven subjects (34%) were slow acetylators. Most of the coloured subjects had the intermediate genotype.

To determine if the genetic variants had an effect on the concentrations of PAS, the results were analysed using pharmacometric methods described in the population PK section.

Table 15 *NAT1* genotype distribution in all the subjects and separated by their ethnic groups

Genotypes (haplotypes)	Genotype distributions		
	All subjects (n=61)	**Coloured subjects (n=48)**	**Black subjects (n=13)**
1*4/1*4	16 (26%)	13 (27%)	3 (23%)
1*10/1*10	10 (16%)	8 (17%)	2 (15%)
1*3/1*10	1 (2%)	1 (2%)	0 (0%)
1*3/1*3	1 (2%)	1 (2%)	0 (0%)
1*4/1*10	27 (44%)	19 (40%)	8 (62%)
1*4/1*27	2 (3%)	2 (4%)	0 (0%)
1*4/1*14A	2 (3%)	2 (4%)	0 (0%)
1*4/1*3	1 (2%)	1 (2%)	0 (0%)
1088AA homozygote	1 (2%)	1 (2%)	0 (0%)

Table 16 *NAT2* genotype distribution in study 2 in all subjects and separated by their ethnic groups

Genotypes (haplotypes)	Genotype distributions		
	All subjects (n= 32)	**Coloured subjects (n=27)**	**Black subjects (n=5)**
F/F (Fast)	7 (22%)	7 (26%)	0 (0%)
F/S (intermediate)	14 (44%)	12 (44%)	2 (40%)
S/S (Slow)	11 (34%)	8 (30%)	3 (60%)

5.5. Population Pharmacokinetics

5.5.1. Excluded and Missing Data

The available data of both studies were used, including the six subjects that were not included in the occasions and regimen comparisons described above. Therefore, forty-one subjects (n=41) from study 1 and thirty two subjects (n=32) from study 2 were used in the modelling and simulation analysis.

Exclusion criteria for data from the analysis were mainly for samples reported below the lower limit of quantification.

5.5.2. Base Model

A one-compartment open model with first-order absorption was fitted to the steady-state concentration-time data of individuals administered 8 g once-daily or 4 g twice-daily GSR-PAS. The structural pharmacokinetic model for the one-compartment model was parameterized on oral clearance (CL), volume of the central compartment (V), absorption rate constant (K_a), lag time (T_{lag}) and oral bioavailability (F) which was fixed at 1. The first twice-daily PAS profile from study 1 was designated as occasion 1, and the second twice-daily profile as occasion 2. The twice-daily PAS profile obtained in study 2 was set as occasion 3 and the once-daily profile as occasion 4. ω_p represents the approximate coefficient of variation for the interindividual variability and π_p is the approximate coefficient of variation for the interoccasion (intraindividual) variability within an individual. A combined proportional and additive residual error model was used. The pharmacokinetic estimates for each individual were subsequently obtained using Bayesian estimation. The final model parameters are shown in Table 17.

The population mean for the volume of distribution (V) was approximately 66.6 L and clearance (CL) was 12.4 L/h. The absorption rate constant was 0.247 h^{-1} and the corresponding lag time was 1.55 h. The interindividual variability for both V and CL was approximately 40%. Interoccasion variability of the CL parameter was approximately 37%. The allometric exponent for the correlation between clearance and body weight was 0.321.

The model diagnostics are shown in Figure 20. There was a good agreement between model predictions and observations of PAS concentrations. The plots of the observed (OBS) concentrations vs. the model predicted (PRED) concentrations and OBS vs. individual predicted (IPRED) concentrations are shown in the top panel of Figure 20. The body weighted residuals (WRES) vs. time and WRES vs. PRED plots at the bottom panel of Figure 20 show that most of the data lie within 2 units from the zero-ordinate. Model stability was evaluated on the basis of 500 bootstrap replicates presented in Table 17. The 2.5th and 97.5th percentiles of the parameter estimates of the final model based on the bootstrap algorithm encompassed the mean population parameter estimates of the final model; the mean values were close to the bootstrap medians.

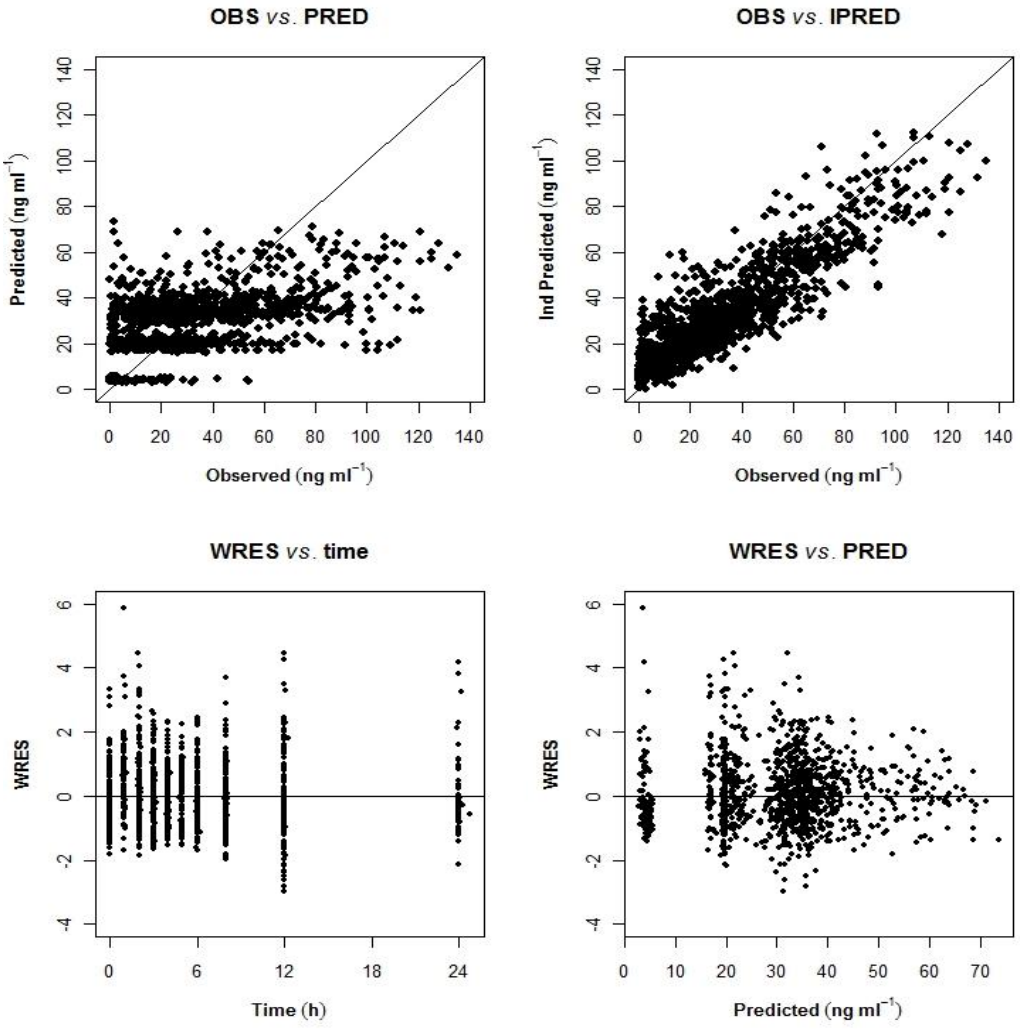

Figure 20 Goodness-of-fit plots for the final GSR-PAS population pharmacokinetic model

Table 17: Population pharmacokinetic model parameters of the final model given no covariate relationship except for weight

Parameter	Final Model		Bootstrap	
	Mean	Standard error	Median	2.5th – 97.5th Percentiles
Structural model parameters				
Clearance (CL), L/h	12.4	1.14	12.8	10.9, 14.9
Volume of central compartment (V), L	66.6	9.61	65.2	43.4, 89.5
Absorption rate constant (K_a), h^{-1}	0.247	0.041	0.240	0.171, 0.348
Lag time (T_{lag}), h	1.55	0.07	1.56	1.43, 1.70
Interindividual variability				
%CV of CL (ω_{CL})	38.3	4.8	36.9	26.5, 45.8
%CV of V (ω_V)	40.8	9.3	40.6	20.9, 71.2
%CV of K_a (ω_{Ka})	72.6	17.2	70.5	16.7, 104
Interoccasion variability				
%CV of CL (κ_{CL})	37.1	5.2	37.0	27.5, 48.4
Residual variability				
Proportional residual error	0.294	0.078	0.300	0.16, 0.40
Additive residual error, IU/dL	8.53	2.03	8.30	5.09, 11.8
Covariates				
Allometric exponent for CL by weight†	0.321	0.343	0.410	0.051, 1.01
OFV	7834.83		7816.38	7462, 8165

†The covariate equations for CL and V by weight (BW) were: $P_i = \tilde{P} \times \left(\frac{BW}{70\ kg}\right)^{\theta_i}$. θ_i for V is fixed to 1

5.5.3. Model Validation

Prior to developing the visual predictive check, the distribution of the drug in the body was simulated assuming normal distribution with a mean value of 56 kg and 11 kg standard deviation, which were the distribution parameters of the body weights of the original subject population. The degenerate visual predictive check results are shown in Figures 21 and 22 for GSR-PAS. The results were generated from 1000 simulations. The 95% prediction interval of the simulated results contained the individual concentration-time profiles by occasion relative to the time of their steady state dose. The simulated profiles were able to capture the majority of the observed data, supporting the validity of the model. These findings indicated that the population estimates in the final model were accurate and stable.

The degenerate visual predictive check (Figure 21 and 22) show the median line in red; the observed drug concentration in black solid circles; and the 95% prediction interval in grey shade of the 1000 simulated profiles for subjects taking GSR-PAS. The top panels are for data in the linear scales and the lower panels are for data in semi-log scales.

The profiles of occasion 1 and 2 are almost identical in the visual predictive check (Figure 21); however the 95% prediction interval (grey shade) is different given that the peak at occasion 2 is lower (flatter) than at occasion 1.

In Figure 22, occasion 4 (once-daily regimen of study 2) has a higher peak than occasion 3 (twice-daily regimen of study 2). These results are similar to the results found in the classical statistical analysis previously presented. The semi-log of the prediction interval for occasion 4 falls below the MIC of 1 µg/ml.

The simulated datasets were used to summarise the mean, median and 5^{th} and 95^{th} percentiles of the exposure parameters in Table 18. The parameters were determined by a non-compartmental method.

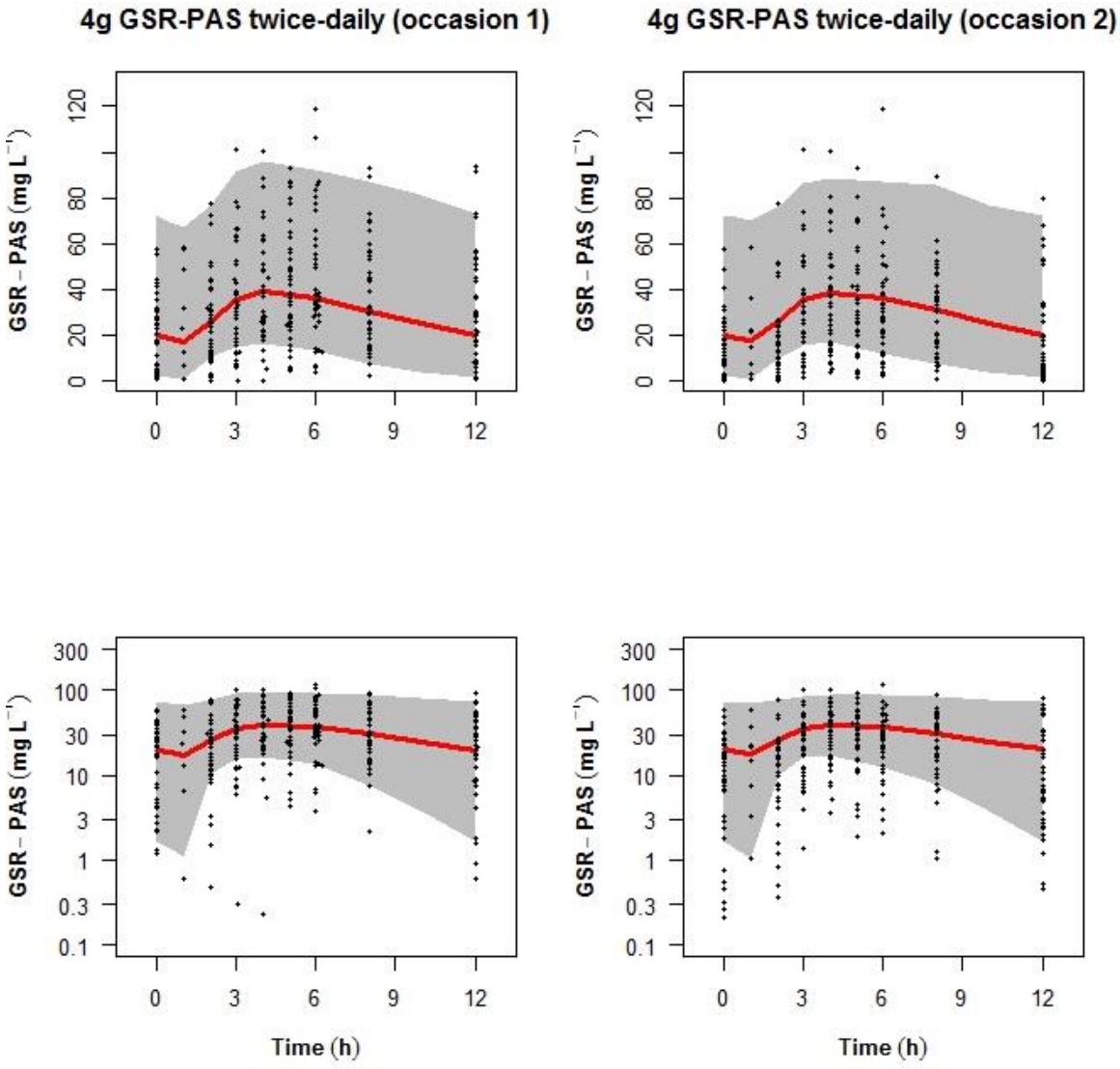

Figure 21 Degenerate visual predictive check results of occasion 1 and 2 (study 1) with weight as a covariate

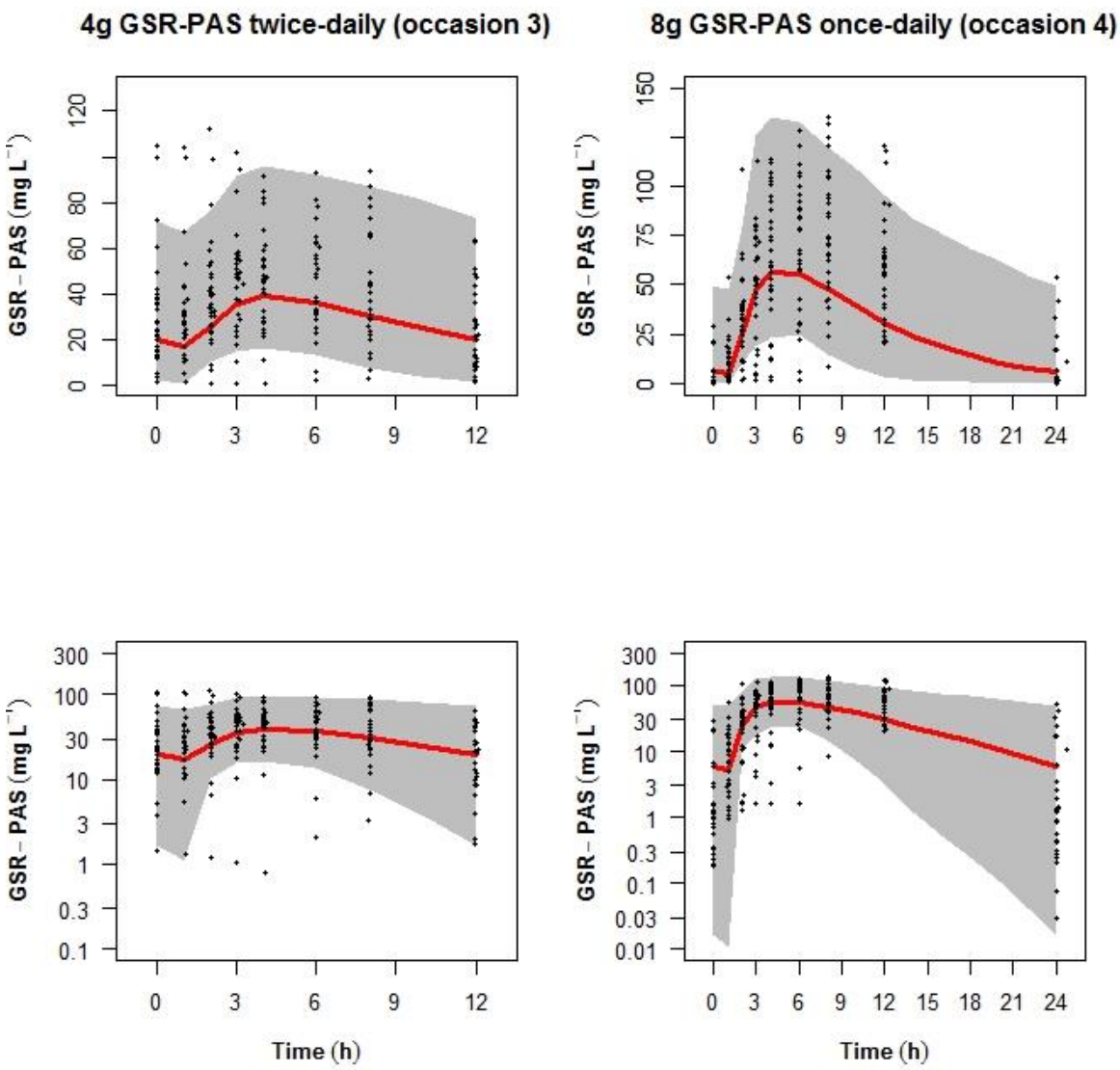

Figure 22 Degenerate visual predictive check results of occasion 3 and 4 (study 2) with weight as a covariate

Table 18 Summary statistics for the pharmacokinetic parameters of GSR-PAS after steady state administration in 4 g twice-daily versus 8 g once-daily regimens in the simulated profiles consisting of 1000 subjects

PK Parameter	Statistics	Dosing Regimens of GSR- PAS		
		4 g twice-daily occasion 1/3 (N=70)	**4 g twice-daily occasion 2 (N=39)**	**8 g once-daily occasion 4 (N=32)**
C_{max} (µg/ml)	Mean	44.8	44.0	67.7
	Median	40.0	39.6	59.8
	5^{th}, 95^{th} percentile	19.0, 88.6	19.7, 82.0	29.3, 128.1
T_{max} (h)	Mean	4.9	4.93	5.74
	Median	4	6	6
	5^{th}, 95^{th} percentile	3, 6	3, 6	3, 8
C_{min} (µg/ml)	Mean	22.0	21.8	9.8
	Median	17.3	17.7	5.1
	5^{th}, 95^{th} percentile	2.2, 56.6	2.2, 57.1	0.04, 35.6
C_{12} (µg/ml)	Mean	24.9	24.0	35.6
	Median	20.5	20.2	30.3
	5^{th}, 95^{th} percentile	3.73, 62.0	2.8, 61.6	6.1, 81.2
C_{24} (µg/ml)	Mean	NA	NA	10.7
	Median	NA	NA	5.9
	5^{th}, 95^{th} percentile	NA	NA	0.06, 37.7
AUC_{12} (µg.h/ml)	Mean	407.4	401.7	NA
	Median	350.8	349.6	NA
	5^{th}, 95^{th} percentile	149.7, 845.1	149.9, 846.2	NA
AUC_{24} (µg.h/ml)	Mean	NA	NA	788.8
	Median	NA	NA	682.8
	5^{th}, 95^{th} percentile	NA	NA	295.2, 1679.2

5.5.4. Drug-drug interactions

A relationship between CYP2B6 substrate and PAS was found during screening for covariates. Efavirenz (EFV) was categorised as the substrate for CYP2B6. EFV increased the clearance of PAS by 45% in subjects using this concomitant drug.

The exponential coefficient for the effect of EFV on PAS CL was 0.37, which translates to 1.45 in the linear scale (Table 19). The increase in clearance is reflected in the generally lower drug concentrations of PAS in subjects who were prescribed EFV, represented by the filled red circles shown in Figures 23 and 24. The degenerate visual predictive check shows the median line in red; the observed drug concentration in black solid circles; and the 95% prediction interval in grey shade of 1000 simulated profiles for subjects taking GSR-PAS. The top panels are for data in the linear scales and the lower panels are for data in semi-log scales.

Using the simulated datasets, the mean, median and 5^{th} and 95^{th} percentiles of the exposure parameters are summarised in Table 20 and 21. Table 20 summarises the parameters of the subjects without concomitant EFV administration and Table 21 those with the concomitant EFV. The parameters were determined by the non-compartmental method. The exposure parameters of PAS without EFV were within the ballpark of those in the pooled dataset in Table 18. With EFV concomitant drugs, the PAS exposure, based on the simulated data, were lower than those without EFV.

Table 19 Population pharmacokinetic model parameters of the final model evaluating drug-drug interaction

Parameter	Final Model		Bootstrap	
	Mean	Standard error	Median	2.5^{th} – 97.5^{th} Percentiles
Structural model parameters				
Clearance (CL), L/h	11.5	1.07	11.6	9.86, 14.03
Volume of central compartment (V), L	39.5	5.81	40.01	29.42, 57.02
Absorption rate constant (K_a), h^{-1}	0.158	0.0118	0.159	0.136, 0.202
Lag time (T_{lag}), h	1.60	0.0604	1.60	1.49, 1.71
Interindividual variability				
%CV of CL (ω_{CL})	35.78	18.33	34.65	25.3, 43.5
%CV of V (ω_V)	66.03	49.90	85.67	39.5, 116.6
%CV of K_a (ω_{Ka})	17.78	19.24	19.73	10.7, 42.4
Interoccasion variability				
%CV of CL (κ_{CL})	36.19	18.81	35.69	26.4, 46.38
Residual variability				
Proportional residual error	0.242	0.05	0.25	0.144, 0.375
Additive residual error, IU/dL	9.89	1.25	9.62	6.12, 12.08
Covariates				
Allometric exponent for CL by weight†	0.427	0.273	0.50	0.071, 1.02
CYP2B6 substrate (efavirenz) on CL‡	0.37	131	0.347	0.12, 0.625
OFV	7819.40		7820.18	7495, 8149

†The covariate equations for CL and V by weight (BW) were: $P_i = \tilde{P} \times \left(\frac{BW}{70\ kg}\right)^{\theta_i}$. θ_i for V is fixed to 1

‡The covariate equation to describe the effect of efavirenz, a CYP2B6 substrate, on CL: $P_i = \tilde{P}\exp(\theta_I \cdot I_{CYP2B6})$.

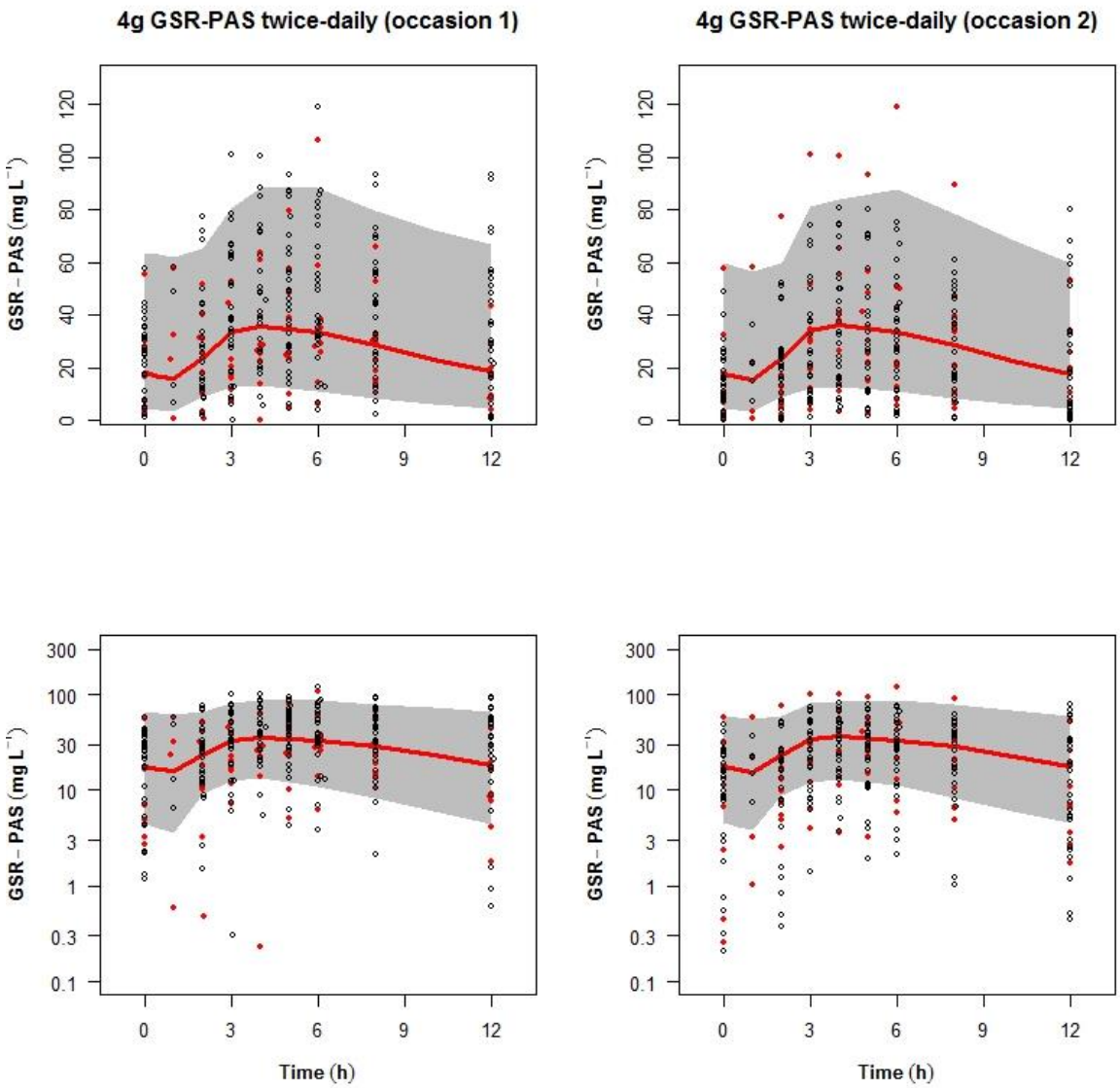

Figure 23 Degenerate visual predictive check results of occasion 1 and 2 (study 1) with weight and EFV drug-interaction as covariates

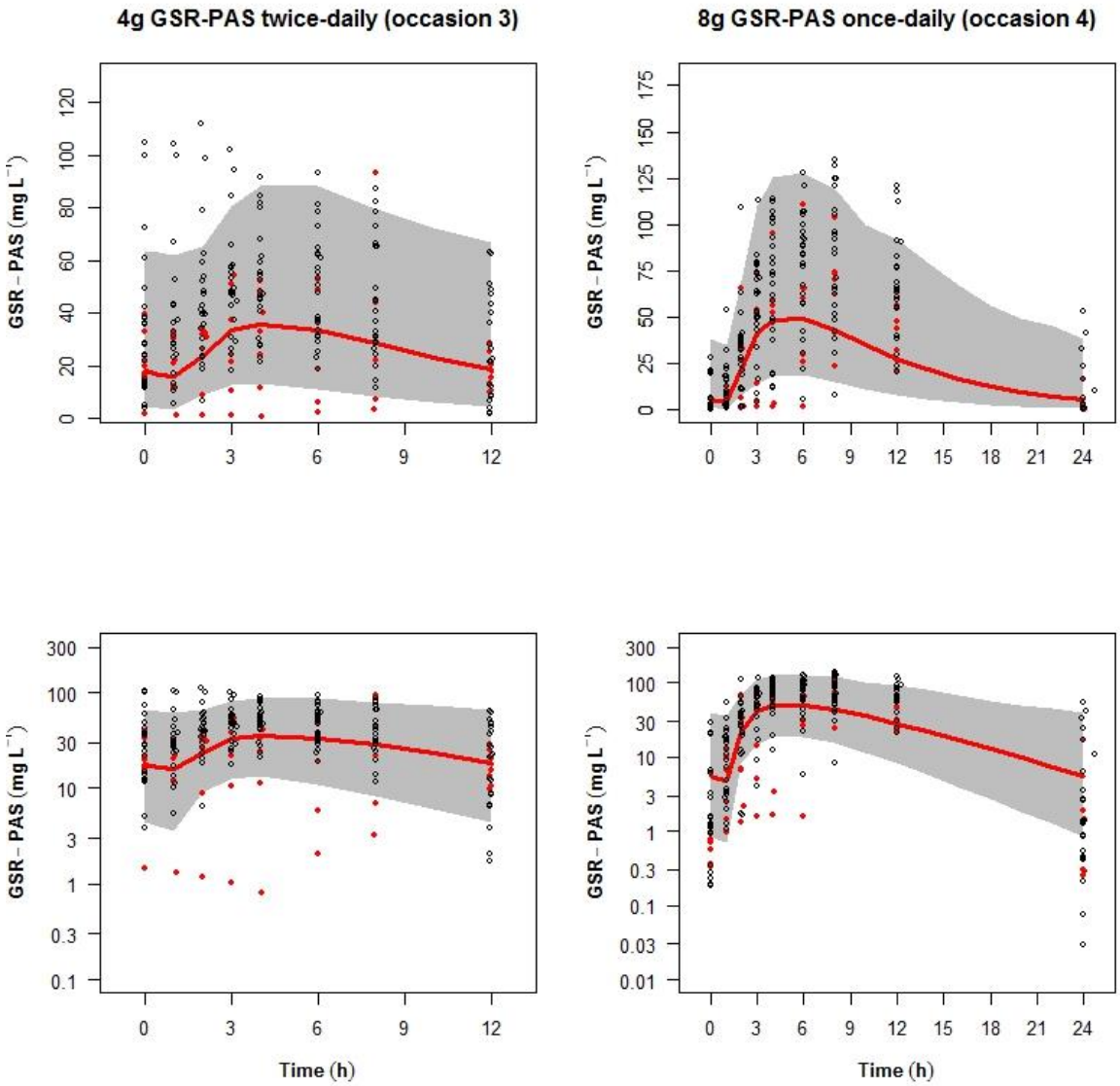

Figure 24 Degenerate visual predictive check results of occasion 3 and 4 (study 2) with weight and EFV drug-interaction as covariates

Table 20 Summary statistics for the pharmacokinetic parameters of GSR-PAS after steady state administration in 4 g twice-daily versus 8 g once-daily regimens in the simulated profiles consisting of 1000 subjects without concomitant efavirenz administration.

PK Parameter	Statistics	Dosing Regimens of GSR- PAS		
		4 g twice-daily occasion 1/3 (N=70)	**4 g twice-daily occasion 2 (N=39)**	**8 g once-daily occasion 4 (N=32)**
C_{max} (µg/ml)	Mean	47.5	45.9	68.1
	Median	43.9	42.3	62.8
	5^{th}, 95^{th} percentile	20.1, 88.3	20.8, 86.9	31.0, 125.7
T_{max} (h)	Mean	5.0	5.0	5.8
	Median	6	6	6
	5^{th}, 95^{th} percentile	3, 6	3, 6	3, 8
C_{min} (µg/ml)	Mean	24.6	23.5	10.4
	Median	20.0	19.8	6.4
	5^{th}, 95^{th} percentile	6.5, 56.7	6.5, 53.0	1.2, 35.1
C_{12} (µg/ml)	Mean	27.2	24.0	40.1
	Median	22.3	20.2	34.1
	5^{th}, 95^{th} percentile	7.7, 60.5	2.8, 61.6	12.8, 89.7
C_{24h} (µg/ml)	Mean	NA	NA	11.5
	Median	NA	NA	7.2
	5^{th}, 95^{th} percentile	NA	NA	1.4, 37.3
AUC_{12} (µg.h/ml)	Mean	442.0	427.0	NA
	Median	384.7	375.7	NA
	5^{th}, 95^{th} percentile	168.8, 864.1	182.2, 847.3	NA
AUC_{24} (µg.h/ml)	Mean	NA	NA	841.6
	Median	NA	NA	738.3
	5^{th}, 95^{th} percentile	NA	NA	345.7, 1694.7

Table 21 Summary statistics for the pharmacokinetic parameters of GSR-PAS after steady state administration in 4 g twice-daily versus 8 g once-daily regimens in the simulated profiles consisting of 1000 subjects with concomitant efavirenz administration.

		Dosing Regimens of GSR- PAS		
PK Parameter	**Statistics**	**4 g twice-daily occasion 1/3 (N=70)**	**4 g twice-daily occasion 2 (N=39)**	**8 g once-daily occasion 4 (N=32)**
C_{max}	Mean	35.1	35.3	54.4
(µg/ml)	Median	31.4	30.7	47.5
	5^{th}, 95^{th} percentile	14.5, 70.4	14.8, 69.8	21.5, 109.6
T_{max}	Mean	4.6	4.8	5.3
(h)	Median	4	4	6
	5^{th} , 95^{th} percentile	3, 6	3, 6	3, 8
C_{min}	Mean	16.1	16.5	6.0
(µg/ml)	Median	12.4	13.1	3.4
	5^{th}, 95^{th} percentile	3.7, 39.8	3.9, 43.0	0.74, 20.4
C_{12}	Mean	18.0	18.4	26.9
(µg/ml)	Median	14.2	14.8	22.8
	5^{th}, 95^{th} percentile	4.5, 43.5	4.6, 46.7	8.6, 58.1
C_{24}	Mean	NA	NA	6.7
(µg/ml)	Median	NA	NA	4.0
	5^{th}, 95^{th} percentile	NA	NA	0.89, 21.9
AUC_{12}	Mean	311.3	316.4	NA
(µg.h/ml)	Median	266.6	267.3	NA
	5^{th}, 95^{th} percentile	137.3, 1004.5	138.7, 1067.8	NA
AUC_{24}	Mean	NA	NA	602.6
(µg.h/ml)	Median	NA	NA	520.4
	5^{th}, 95^{th} percentile	NA	NA	244.1, 1165.9

5.5.5. Effect of *NAT1* and *NAT2* polymorphisms

The influence of *NAT1* and *NAT2* gene variants on the disposition of PAS was initially inspected by plotting the time-concentration profiles of each genotype against other genotypes combined, as shown in Figures 25 - 27 and 29. These graphs were generated to depict if there are any associations between the concentrations and the different genotypes. The specific genotype is shown in red and the rest in black. There is a wide variation in the concentrations of the specific genotypes. The concentrations of the single 8 g dose were also plotted with the concentrations of the 4 g dose. Therefore, in order to separate the two different doses, the mean PAS pharmacokinetic profiles of the different *NAT1* genotypes for the 4 g were generated and depicted in Figure 28. Since only three subjects had the respective *NAT1*3/1*3, NAT1*3/1*10* and *NAT1*4/1*3* genotypes, these genotypes were not included in the graph.

The *NAT1*4/1*4* is the wild type and is considered as a fast acetylator. In theory the individuals with this genotype will have lower PAS plasma concentrations due to an increase in acetylating activity. The mean PAS plasma concentrations of the *NAT1*4/1*4* are lower than the other genotypes (Figure 28); however this difference is only small. Two subjects had the *NAT1*4/1*14A* genotype and their mean PAS plasma concentrations were higher than the other genotypes. Although the concentrations of the NAT1*4/1*14A are higher, the sample size (two subjects) are too small to find any significance. The *NAT1*10/1*10, NAT1*4/1*10, NAT1*4/1*4* and *NAT1*4/1*27* had low concentrations.

The individuals with the *NAT1*4/1*4* genotype were designated as the baseline, the reference genotype to which the other genotypes were compared. No significance was found between the concentrations of the different genotypes (possibly due to small sample size), therefore to increase the size of the comparison, individuals with *NAT1*4/1*10, NAT1*4/1*14A* and *NAT1*10/1*10* genotypes were grouped together, as a comparator to the wild-type. Due to their small sample size, the following genotypes were grouped with the wild-type group: *NAT1*4/1*27, NAT1*3/1*3, NAT1*4/1*3* and *NAT1*3/1*10*. For the individuals whose *NAT1* genotype was not determined, the patterns were similar to that for *NAT1*4/1*4* and therefore were also grouped with the wild-type.

Dummy variable were introduced to the *NAT1* categories. The wild-type group were designated as 0 whereas the group consisting of *NAT1*4/1*10, NAT1*4/1*14A* and *NAT1*10/1*10* genotypes were designated as 1. The effect of the second group on PAS clearance was quantified as a decrease of approximately 20%, compared to the wild-type (Table 22).

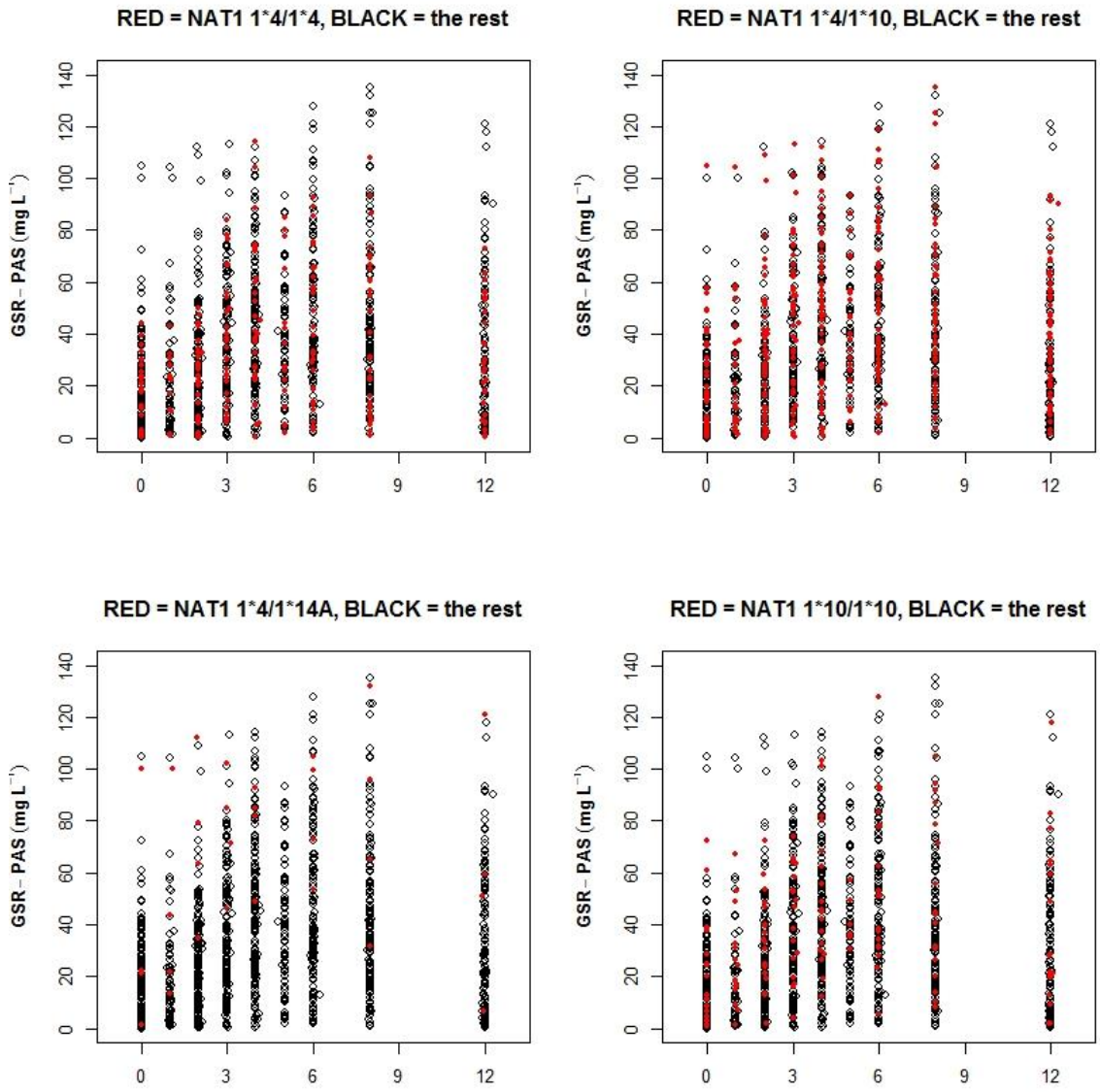

Figure 25 Visual diagnostics to inspect the influences of *NAT1*4/1*4, NAT1*4/1*10, 1*4/*14A* and *NAT1*10/1*10* on the disposition of PAS

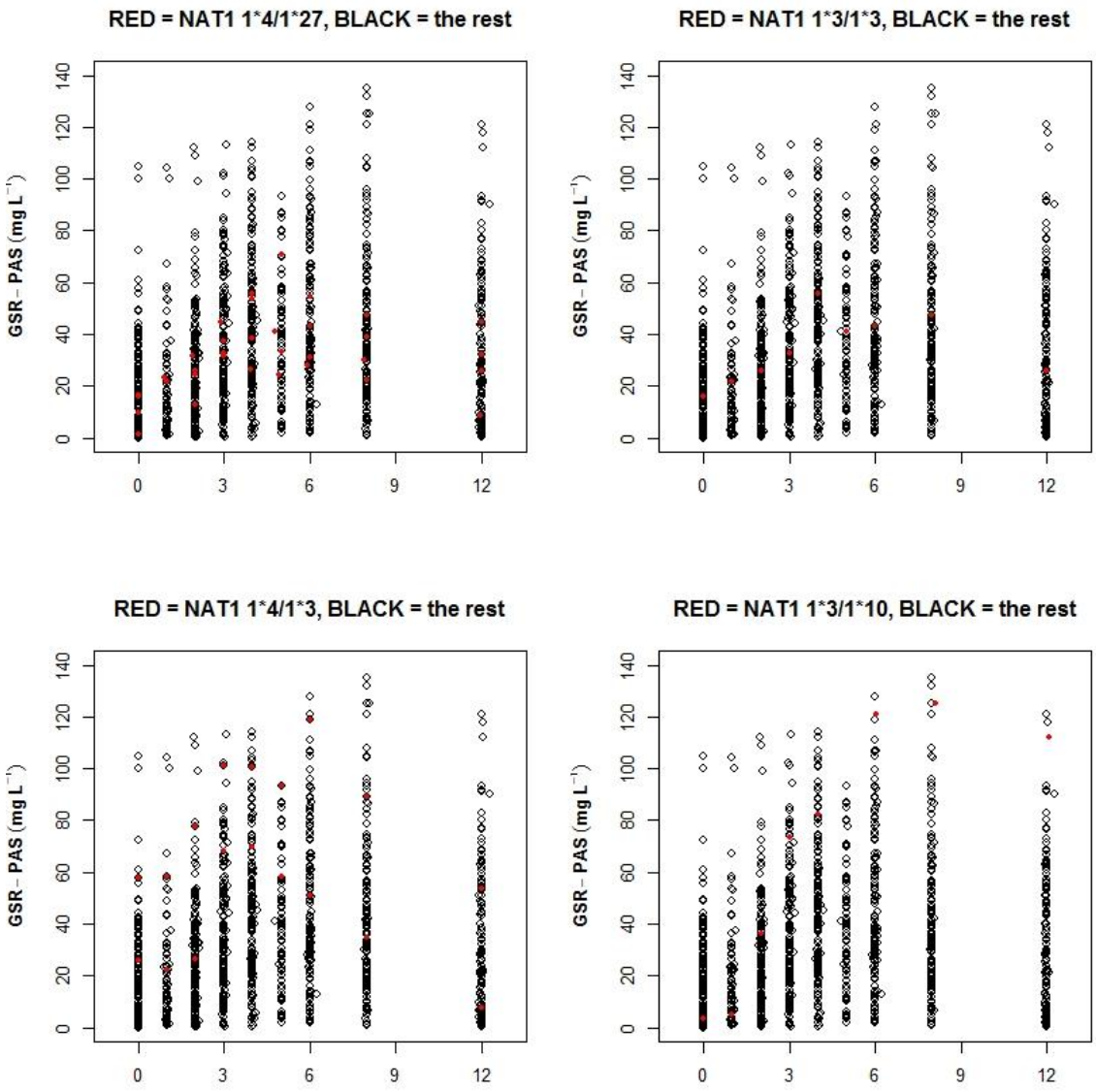

Figure 26 Visual diagnostics to inspect the influences of *NAT1*4/1*27, NAT1*3/1*3, NAT1*4/*3* and *NAT1*3/1*10* on the disposition of PAS

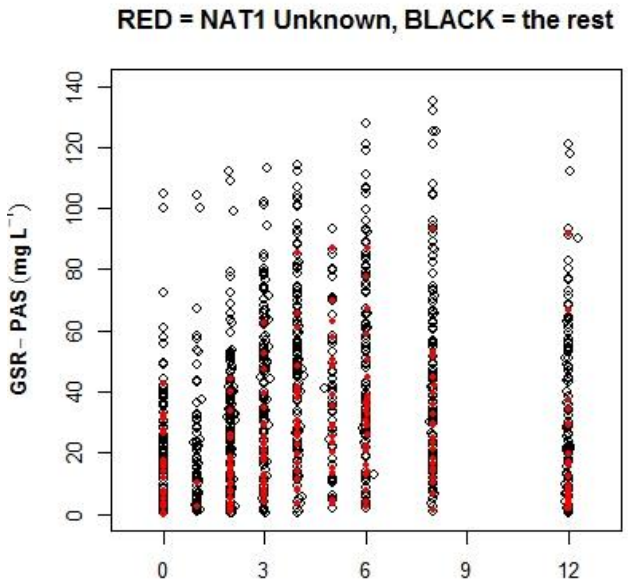

Figure 27 Visual diagnostics to inspect the disposition of PAS in individuals who do not have their *NAT1* genotype identified.

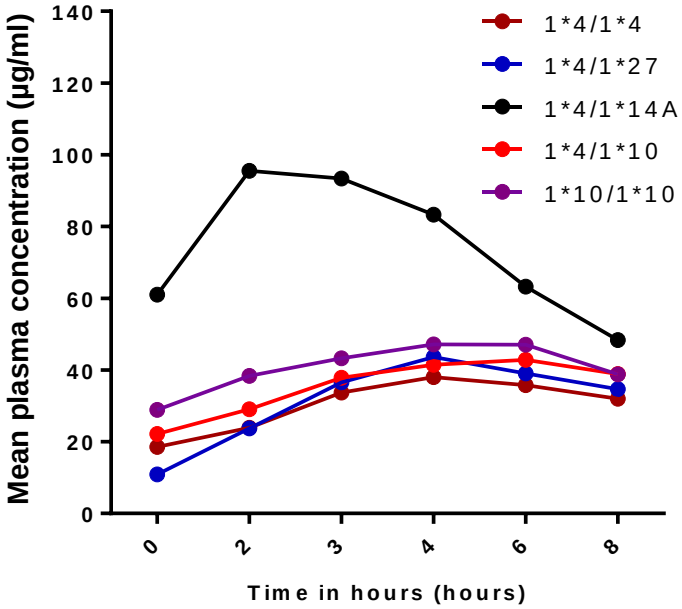

Figure 28 Mean PAS concentrations of the different *NAT1* genotypes after administration of 4 g GSR-PAS

Table 22 Population pharmacokinetic model parameters of the final model evaluating drug-drug interaction and *NAT1* genotype influence.

Parameter	Final Model		Bootstrap	
	Mean	Standard error	Median	2.5th – 97.5th Percentiles
Structural model parameters				
Clearance (CL) L/h	12.8	1.38	13.1	10.63, 16.63
Volume of central compartment (V), L	39.5	6.07	40.9	29.26, 62.96
Absorption rate constant (K_a), h^{-1}	0.158	0.0127	0.160	0.140, 0.209
Lag time (T_{lag}), h	1.60	0.0605	1.60	1.48, 1.71
Interindividual variability				
%CV of CL (ω_{CL})	33.76	18.33	34.65	25.3, 43.5
%CV of V (ω_V)	85.73	49.90	85.67	39.5, 116.6
%CV of K_a (ω_{Ka})	18.14	19.24	19.73	10.7, 42.4
Interoccasion variability				
%CV of CL (κ_{CL})	36.47	18.81	35.69	26.4, 46.38
Residual variability				
Proportional residual error	0.244	0.0516	0.244	0.147, 0.386
Additive residual error, IU/dL	9.84	1.30	9.56	5.88, 12.22
Covariates				
Allometric exponent for CL by weight†	0.334	0.195	0.382	0.047, 0.935
CYP2B6 substrate (efavirenz) on CL‡	0.323	0.135	0.312	0.026, 0.576
***NAT1* on CL††**	0.199	0.0905	0.208	-0.022, 0.36
OFV	7815.318		7798.47	7430, 8143

†The covariate equations for CL and V by weight (BW) were: $P_i = \tilde{P} \times \left(\frac{BW}{70\ kg}\right)^{\theta_i}$. θ_i for V is fixed to 1

‡The covariate equation to describe the effect of efavirenz, a CYP2B6 substrate, on CL: $P_i = \tilde{P}\exp(\theta_I \cdot I_{CYP2B6})$.

††The covariate equation to describe the effect of *NAT1* genotype on CL: $P_i = \tilde{P}(1 - \theta_I \cdot I_{NAT1})$.

The *NAT2* genotypes and phenotypes could only be determined in subjects from the second study. Their influences on PAS disposition are presented graphically in Figure 29.

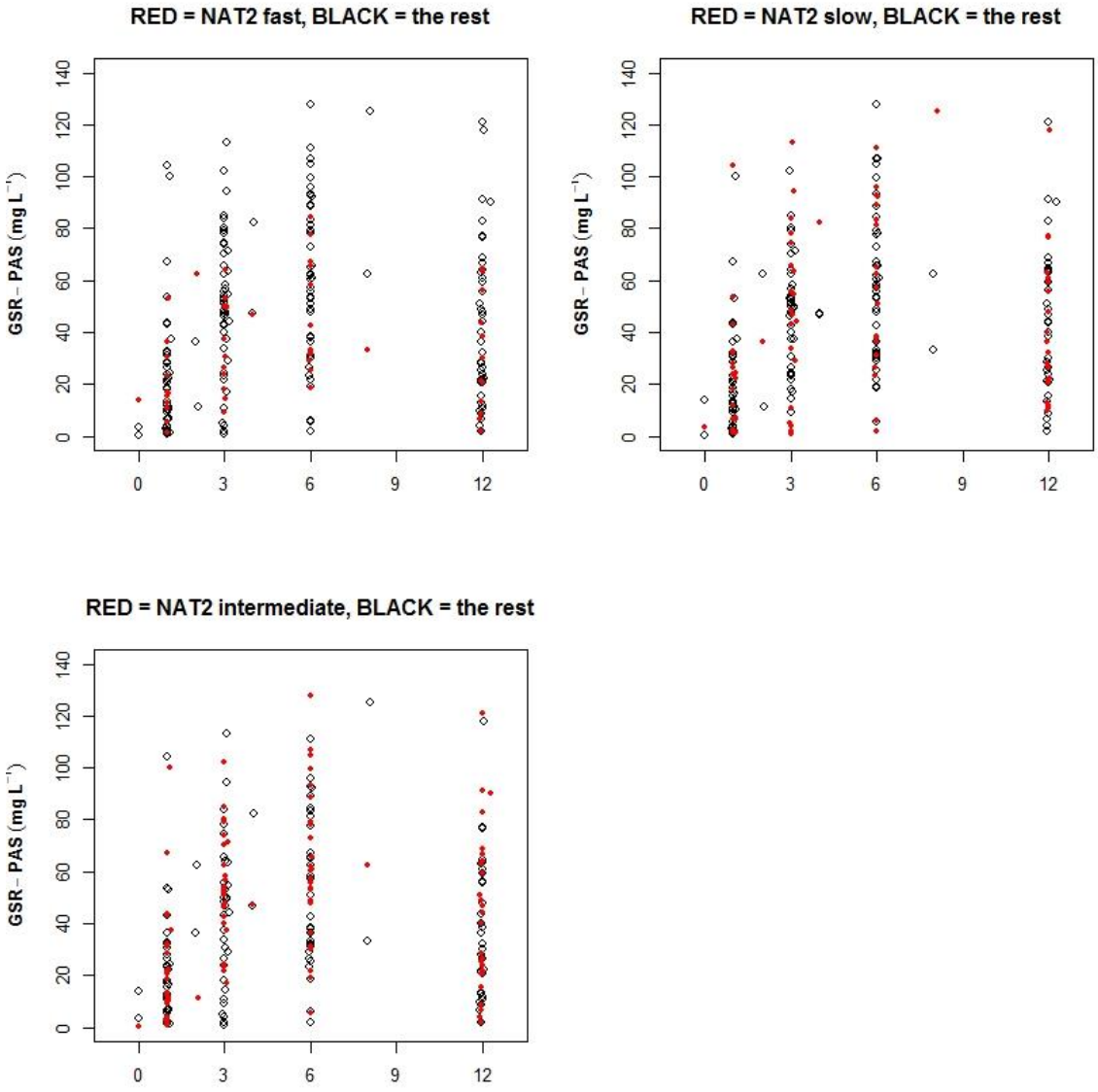

Figure 29 Visual diagnostics to inspect the influences of *NAT2* phenotypes on the disposition of PAS.

The *NAT2* does not play a major role in PAS metabolism [17]. However, several studies reported interaction between *NAT1* and *NAT2* polymorphisms [100]. An interaction plot was generated to evaluate whether there is a trend that would suggest an interaction. In Figure 30, *NAT1*4* heterozygous and *NAT1*10* heterozygous in the fast-intermediate *NAT2* phenotypes had a relatively slower clearance than in the slow *NAT2* phenotype. This interaction, however, was not statistically significant, suggesting that *NAT2* phenotype has no effect on PAS disposition.

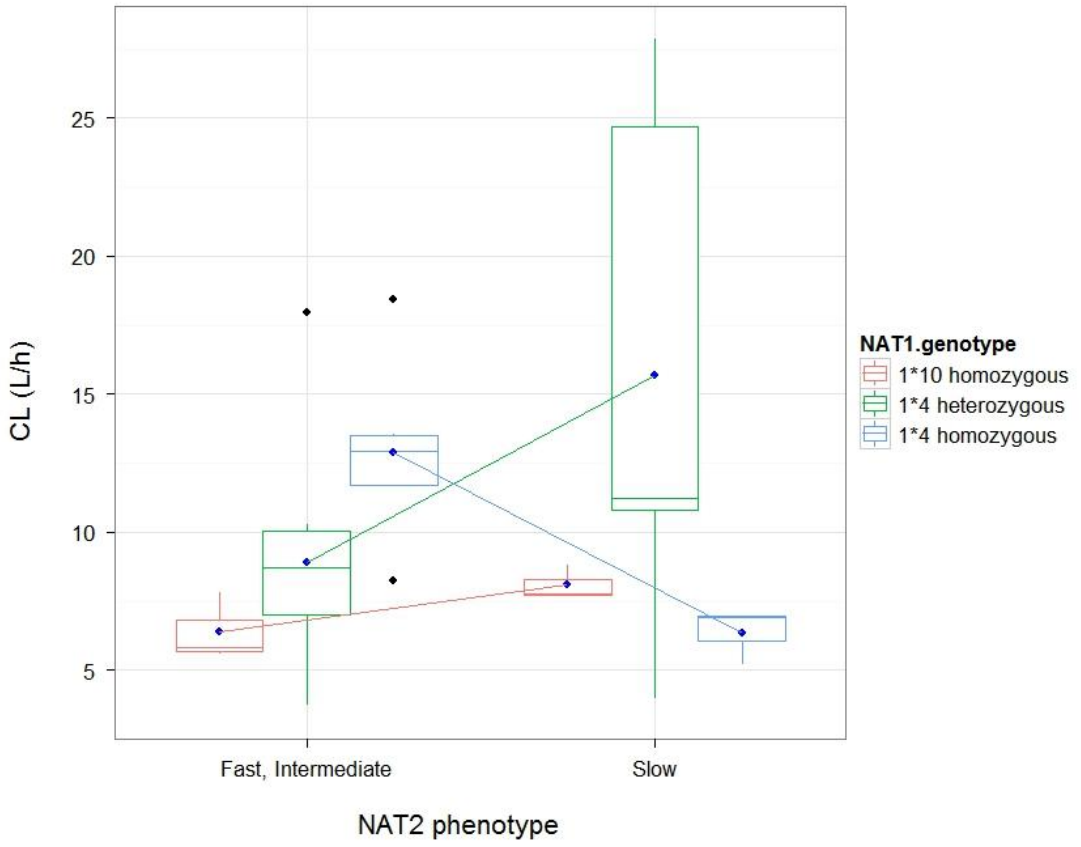

Figure 30 Interaction plot evaluating the trend in *NAT1* genotype and *NAT2* phenotypes and their effect on clearance

5.6. Study 2: Tolerability and safety

Tolerability and safety of the two different treatment regimens were monitored in study 2. All subjects completed the VAS forms which specifically recorded gastrointestinal adverse reactions. Mean scores of gastrointestinal intolerabilities for each day were calculated and summarised in Figures 31-35 and in Table 23.

Abdominal pain and cramps

According to the VAS scores, eleven subjects did not have any abdominal pains or cramps, while nine subjects only experienced such symptoms during the twice-daily regimen. Nine subjects reported abdominal pain and cramps during both regimens. Five of the nine subjects reported abdominal pain and cramps as worse during the once-daily regimen. The other four subjects gave a higher score for abdominal pain and cramps during the twice-daily regimen. On average, the VAS scores for abdominal pain and cramps were higher during the twice-daily regimen. Figure 31 illustrates the mean VAS scores for abdominal pain and cramps experienced during the study.

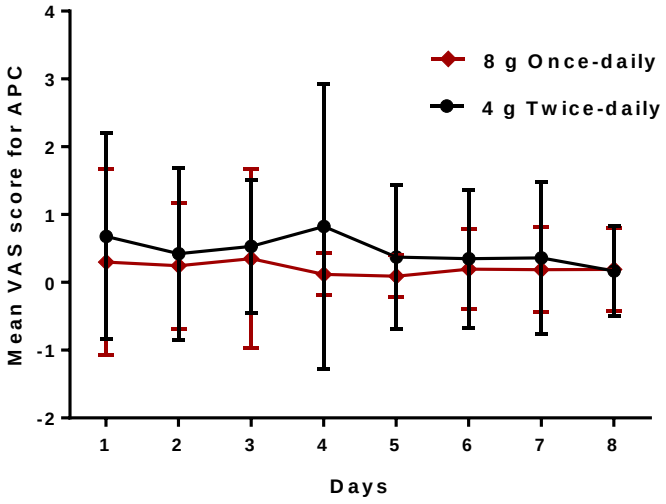

Figure 31 Comparison of the mean VAS scores for abdominal pain and cramps (APC) over 8 days during 8 g once- and 4 g twice-daily administration of GSR-PAS

Nausea

According to the VAS scales only two subjects did not report nausea during the study. Four subjects reported nausea only during the once-daily regimen, six subjects during the twice-daily regimen, and seventeen subjects during both regimens. Ten of these latter subjects gave a higher score for nausea during the once-daily regimen and seven subjects during the twice-daily regimen. Figure 32 illustrates the mean VAS scores for nausea in the study. Nausea varied on a daily base for the twice-daily regimen, while nausea for the once-daily regimen was more constant.

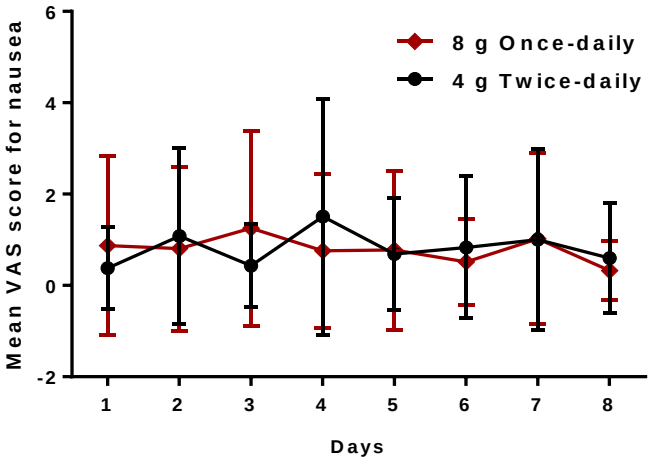

Figure 32 Comparison of the mean VAS scores for nausea over 8 days during 8 g once- and 4 g twice-daily administration of GSR-PAS

Vomiting

According to the VAS scores fifteen of the subjects did not vomit during the study. Two subjects vomited during the once-daily regimen, four during the twice-daily regimen, and the remaining eight subjects during both regimens. Four of the former group experienced more vomiting during the once-daily regimen and three subjects during the twice-daily regimen. One subject vomited once during both regimens. On average, the VAS scores for vomiting were higher during the twice-daily regimen. Figure 33 illustrates the mean VAS scores for vomiting. Vomiting was similar for the once- and twice-daily regimens. The mean score on Day 3 for the once-daily regimen

was influenced by one subject who had an episode of severe vomiting in the morning, which cleared up later.

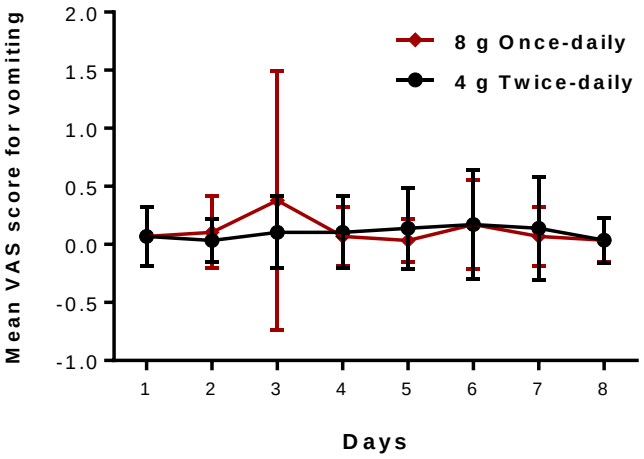

Figure 33 Comparison of the mean VAS scores for vomiting over 8 days during 8 g once- and 4 g twice-daily administration of GSR-PAS

Diarrhoea

According to the VAS scores seven subjects did not have any diarrhoea during the study. One subject had diarrhoea only during the once-daily regimen, two subjects only during the twice-daily regimen and nineteen subjects during both regimens. Twelve of these latter subjects had more diarrhoea during the once-daily regimen and seven subjects during the twice-daily regimen. Figure 34 illustrates the mean VAS scores for diarrhoea during the study. On average, the VAS scores for diarrhoea were higher during the once-daily regimen. Diarrhoea was constant for the twice-daily regimen throughout the 8 days.

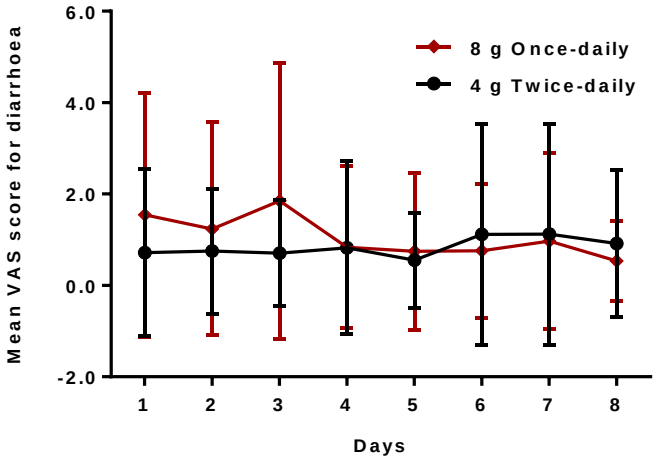

Figure 34 Comparison of the mean VAS scores for diarrhoea over 8 days 8 g once- and 4 g twice-daily administration of GSR-PAS

Bloatedness

According to the VAS scores ten subjects did not report the symptom "bloatedness" in the study. Five subjects reported this symptom only during the twice-daily regimen, two subjects during the once-daily regimen and twelve subjects during both regimens. On average, the VAS scores for bloatedness were higher during the twice-daily regimen. Figure 35 illustrates the mean VAS scores for bloatedness.

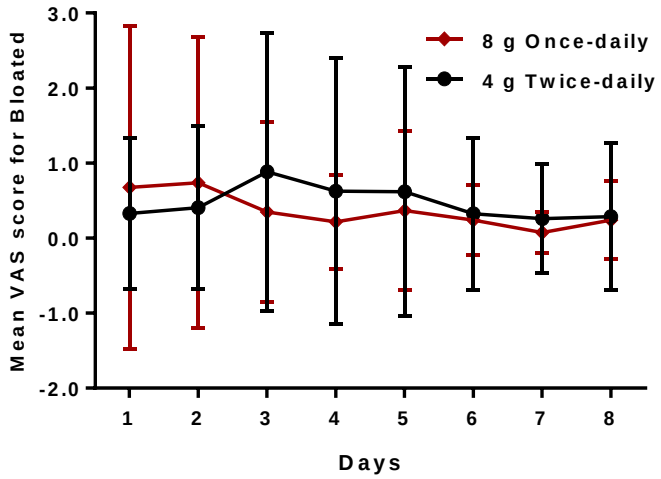

Figure 35 Comparison of the mean of the VAS scores for bloating over 8 days during 8 g once- and 4 g twice-daily administration of GSR-PAS

The VAS scores for each day were analysed for significant differences using the Wilcoxon rank sum test. The results are summarised in Table 23.

There were no significant differences between the once-daily and twice-daily regimen for tolerability, except for abdominal pain on Day 3 and diarrhoea on Day 1. It cannot be excluded that this may be a chance finding due to the high number of statistical tests performed.

Table 23 Summary of gastrointestinal symptoms (abdominal pain and cramps, nausea) for the 8 g once-daily vs. 4 g twice-daily regimens

Gastrointestinal Symptoms	**Once-daily Mean; SD**	**Twice-daily Mean; SD**	**p-value**
Abdominal Pain and Cramps			
Day 1	0.3; 1.37	0.68; 1.52	0.327
Day 2	0.25; 0.93	0.42; 1.27	0.683
Day 3	0.35; 1.32	0.53; 0.98	0.035
Day 4	0.12; 0.31	0.83; 2.10	0.187
Day 5	0.09; 0.31	0.37; 1.06	0.337
Day 6	0.19; 0.59	0.35; 1.02	0.559
Day 7	0.19; 0.63	0.36; 1.12	0.280
Day 8	0.19; 0.61	0.17; 0.66	0.598
Nausea			
Day 1	0.87; 1.95	0.38; 0.90	0.288
Day 2	0.81; 1.80	1.08; 1.93	0.768
Day 3	1.25; 2.14	0.43; 0.91	0.085
Day 4	0.76; 1.69	1.51; 2.58	0.146
Day 5	0.78; 1.74	0.69; 1.22	0.666
Day 6	0.52; 0.94	0.83; 1.56	0.493
Day 7	1.02; 1.88	1.01; 1.98	0.866
Day 8	0.33; 0.64	0.60; 1.20	0.314

* Indicates significance ($p < 0.05$); **SD** standard deviation; **CV** coefficient of variation

Table 23 Summary of gastrointestinal symptoms (vomiting, diarrhoea and bloatedness) for the 8 g once-daily vs. 4 g twice-daily regimens (continued)

Gastrointestinal Symptoms	Once-daily Mean; SD	Twice-daily Mean; SD	p-value
Vomiting			
Day 1	0.07; 0.26	0.07; 0.26	0.921
Day 2	0.10; 0.31	0.03; 0.19	0.400
Day 3	0.38; 1.12	0.10; 0.31	0.134
Day 4	0.07; 0.26	0.10; 0.31	0.665
Day 5	0.03; 0.19	0.14; 0.35	0.142
Day 6	0.17; 0.38	0.17; 0.47	0.913
Day 7	0.07; 0.26	0.14; 0.44	0.617
Day 8	0.04; 0.19	0.04; 0.19	1.000
Diarrhoea			
Day 1	1.55; 2.67	0.72; 1.83	0.048
Day 2	1.24; 2.33	0.75; 1.37	0.246
Day 3	1.85; 3.02	0.71; 1.16	0.317
Day 4	0.83; 1.77	0.82; 1.89	0.508
Day 5	0.74; 1.72	0.55; 1.04	0.471
Day 6	0.76; 1.47	1.11; 2.42	0.336
Day 7	0.97; 1.93	1.12; 2.42	0.852
Day 8	0.53; 0.88	0.92; 1.61	0.579
Bloatedness			
Day 1	0.67; 2.15	0.33; 1.00	0.979
Day 2	0.67; 1.93	0.41; 1.09	0.482
Day 3	0.35; 1.20	0.88; 1.86	0.377
Day 4	0.22; 0.63	0.63; 1.77	0.688
Day 5	0.35; 1.06	0.62; 1.66	0.776
Day 6	0.22; 0.46	0.33; 1.01	0.726
Day 7	0.07; 0.28	0.26; 0.73	0.505
Day 8	0.22; 0.51	0.29; 0.98	0.380

* Indicates significance ($p < 0.05$); **SD** standard deviation; **CV** coefficient of variation

VAS scores were compared for individuals with low and high C_{max} values of PAS to investigate if any of these symptoms were concentration-related. Table 24 summarises the mean VAS scores for patients with low and high C_{max} values. Concentrations above 80 µg/ml were considered as high. The maximum concentrations were sub-categorised as below or above 80, 90 and 100 µg/ml.

Scores for abdominal pains and diarrhoea were higher in subjects with maximum concentrations below 80 and 90 µg/ml, while the subjects with maximum concentrations above 80 and 90 µg/ml gave a higher score to vomiting, nausea and bloatedness. The scores of all the gastrointestinal intolerance except for vomiting were higher for the subjects with maximum concentrations below 100 µg/ml. This mixed depiction does not show definite trends and is thus not suggestive of dose-related toxicity.

Table 24 Summary of the gastrointestinal tolerability of individuals with low and high maximum PAS concentrations

Gastrointestinal Symptoms	**Mean VAS scores**	
	≥80 (n=20)	<80 (n=38)
Abdominal pain and cramps	0,272	0,369
Nausea	0,822	0,790
Vomiting	0,119	0,106
Diarrhoea	0,692	1,082
Bloated	0,470	0,371
	≥90 (n=16)	<90 (n=42)
Abdominal pain and cramps	0,230	0,376
Nausea	0,852	0,782
Vomiting	0,148	0,097
Diarrhoea	0,549	1,099
Bloated	0,549	0,351
	≥100 (n=13)	<100 (n=45)
Abdominal pain and cramps	0,071	0,412
Nausea	0,677	0,837
Vomiting	0,135	0,104
Diarrhoea	0,573	1,050
Bloated	0,278	0,442

The VAS scales documented the gastrointestinal intolerances generally associated with PAS. Other reported side effects were documented separately. Reported side effects were loss of appetite (5 of 29, 17%) and tiredness (8 of 29, 28%).

5.7. Study 2: Post-study interview

After the study, a short interview was held with each subject to discuss their experiences. Table 25 summarises the subjects' experience and preference. Thirteen subjects (45%) believed that there was no difference between the two regimens, but ten of them (77%) preferred the twice-daily regimen. The reason provided by the subjects was the bulkiness of the drug when given as a single daily dose in the morning after which they did not have any appetite for breakfast afterwards. Sixteen subjects (55%) claimed that they did not like the once-daily regimen, because of diarrhoea and vomiting. Three subjects (10%) preferred the once-daily administration. According to two subjects, they felt healthier and had more energy. One subject asked if the once-daily regimen could be continued after the study.

5.8. Study 2: Follow-up

Five subjects (16%) were discharged from the hospital. Four subjects (13%) were non-compliant to their TB treatment and failed treatment. One XDR-TB subject (3%) developed hypothyroidism, approximately a month after the study and two subjects (6%) developed renal impairment approximately one to two week after the study. One subject (3%) developed soft tissue inflammation and one subject died (reported as serious adverse events not associated with the study). The other subjects (56%) were in a good condition.

5.9. Study 2: Serious adverse events

One HIV negative subject with XDR-TB completed the study but died with acute renal failure (creatinine level of 1255 µmol/L) two weeks after completion. No abnormalities were reported during and at the follow-up, a week after the study.

Table 25 Summary of the subjects experience and preference of the two regimens in study 2

		Worse Experience		Preferred Regimen	
Subjects	**No difference**	**Once-daily**	**Twice-daily**	**Once-daily**	**Twice-daily**
1	X				X
2	X				X
3	X				X
4	X				X
5		X			X
6		X			X
7		X			X
8		X			X
9		X			X
10	X			X	
11		X			X
12	X				X
13	X				X
14		X			X
15		X			X
16	X			X	
17		X			X
18		X			X
19	X				X
20		X			X
21	X				X
22		X			X
23		X			X
24		X			X
25		X			X
26		X			X
27	X			X	
28	X				X
29	X				X
Total	13 (45%)	16 (55%)	0 (0%)	3 (10%)	26 (90%)

Chapter Six: Discussion

PAS is an old drug and has been formulated in a new granular slow release form i.e. GSR-PAS. Although the benefit of this drug is limited, it is still used as part of a treatment regimen for M/XDR-TB. Since only a few *Mycobacterium* isolates have developed resistance to PAS, it is valuable in preventing resistance to other susceptible drugs.

Even though newer anti-TB agents (e.g. bedaquiline and PA-824 [101,102]) for MDR-TB have been developed in the last few years, PAS may be still useful in preventing the development of resistance to these drugs and may be important in future regimens. However, little is known about its drug interactions, pharmacogenetics and pharmacokinetics.

The recommended dose for PAS is 12 g in divided doses, but 4 g twice-daily has been evaluated and was found to provide adequate concentration for bacteriostasis [81]. However, an ideal treatment regimen in a non-compliant and resource-poor setting is one in which medication is taken only once daily.

Not only has once-daily PAS dose been reported to be clinically as effective [82] as divided daily doses but it also has the potential for early bactericidal activity with a single high dose [103]. These studies, however, used PAS salt formulations, rather than the free PAS encapsulated in granules. Peloquin et al. [81,104] was the only group that has investigated the pharmacokinetics of GSR-PAS and the only group thus far that compared once- and twice-daily GSR-PAS regimens in adults, while Liwa et al [78, 98] specifically focused on children.

The present study provided a comprehensive overview of the pharmacokinetics of PAS in a DR-TB population, in which some subjects were also co-infected with HIV/AIDS. The study focused on several aspects of the clinical pharmacology of PAS including dosing, potential drug interactions and genetic variants. Several unique and novel findings were described for GSR-PAS. Not only did the results indicate that a single 8 g GSR-PAS dose can sustain the PAS plasma concentrations above the

MIC, but also that ART, possibly due to EFV interactions, increases the PAS clearance by 45%.

The additional unpublished data served as a baseline for the study and was therefore incorporated in the analysis and model development. Pharmacometric analysis (population pharmacokinetics) was a supportive tool to explore the data and predict future results. The analysis estimated the population PK parameters and assessed covariates that may change the parameters of the PAS profiles.

Pharmacokinetics

Serial blood samples provided high-quality pharmacokinetic profiles. Wide inter- and intraindividual pharmacokinetic variability was noted in both studies. Several factors can contribute to these variations such as intestinal motility, gastric pH, gastric emptying time, different foods, intestinal metabolism, concomitant drugs, genetic factors, presence of gastrointestinal disease; and prolonged period of absorption that overlaps distribution and excretion [69, 78,104].

The twice-daily regimen of both studies had mean concentrations ranging between 20-60 µg/ml and was thus within the range previously reported for adults also receiving the GSR-PAS formulation [11, 78, 81,104].

The mean PAS concentrations of the twice-daily regimen in study 2 were much higher than the concentrations detected in study 1. The subjects in study 2 received a 12-hourly GSR-PAS dose, while most of the subjects in study 1 received a GSR-PAS dose 15 hours before the next dose. Subsequently, lower PAS plasma concentrations were detected in the pre-dose samples (C_0) of study 1 (Table 12). The PAS concentration detected in the C_0 is the starting concentration of the following dose and therefore a high C_0 can increase the PAS plasma concentration as noted in study 2 (Table 13). In addition, interindividual variability could also have contributed to this finding.

Study 1: Two occasions (4 g twice-daily)

PAS plasma concentrations in study 1 were significant lower during the second than during the first occasion. The same finding had been reported by Liwa [98]. This tendency for the subjects to have lower PAS concentration levels in occasion 2 (2-4 weeks after the first occasion) can be due to the significant increase in body weight,

which is associated with a decrease in plasma concentrations. However, the mean body weight between the two occasions would only explain a difference in concentrations of 2%.

PAS PK profiles are highly variable and as mentioned various factors as well as the change in disease state, concomitant drugs and different diets between the two occasions (2-4 weeks apart), can contribute to these variations.

The median lines (red lines) in the visual predictive graphs of the two occasions (Figure 21) were almost identical, indicating that the difference between the mean PK graphs may be due to larger or smaller outlier values. The 95% predictive interval for occasion 2 was lower than occasion 1. However, when the parameters for twice-daily regimens of 1000 subjects were compared, the results showed only a small value difference.

Study 2: Once vs. Twice-daily

As expected, dose proportionality was seen between the single 8 g daily dose and the 4 g twice-daily dose (Figure 17). The 8 g daily dose generated a mean AUC_{12} that was larger and, a mean C_{max} that was much higher and later than the 4 g dose. Not only did the 8 g dose have a significant higher C_{max} and later peak, but also an earlier start. The same pattern was reported by Liwa et al [78] in children receiving a single dose of 150 mg/kg and a twice-daily dose of 75 mg/kg. The difference between the mean maximum concentrations of the 150 mg/kg and 75 mg/kg doses was however, much smaller in the children's study. The earlier start for the single high dose (8 g for adults and 150 mg/kg for children) could be due to the release and absorption of PAS from the granules in the stomach.

PAS is mainly released and absorbed in the more alkaline intestines[12]. Small amounts however, can also be absorbed in the stomach if the free PAS is unprotected by enteric coating. The free PAS can be released from the granules if the pH of the stomach is increased due to consumption of more alkaline food or beverages shortly after PAS administration [12]. The free PAS can also be exposed if the granules are chewed, which was often noticed in the higher dose. In addition, more granules (higher dose) are exposed for potential absorption through the intestines, which will result in an earlier rise in concentration.

A t_{max} of GSR-PAS normally ranges between 4-8 hours [11]. Although the later peak seems to be normal, the t_{max} of the once-daily regimen was clearly later compared to the twice-daily regimen. The later peak seen in the once-daily regimen can be outlier values. However, the mean t_{max} of the once-daily regimen (Table 13) is less than the median, which indicates that most of the maximum concentrations were at a later period.

A later peak was also visible in the simulated profiles (Table 18) of the two regimens, but in contrast to the original data, the difference was much smaller. With the predicted simulated profiles, the t_{max} of the once-daily regimen had a 90% CI of (3, 8), indicating that once-daily regimen will have patients with a peak that is two hours later than the twice-daily regimen which has a 90% CI of (3, 6).

The later peak observed for the once-daily regimen is unusual, since the once-daily regimen had an earlier start with a much lower starting concentration (C_0) than the twice-daily regimen. GSR-PAS formula is specifically designed to slowly release the PAS from the granules and may therefore be involved in the later peak observed for the higher dose. Walter and Heilmeyer[105] described that the later C_{max} is dose related; therefore, as the dose increases, the time will also increase.

With a bacteriostatic agent the aim is to maximise the time the drug concentration is above the MIC. Since PAS is thought to be mainly a bacteriostatic agent, the aim was to investigate if the different regimens could maintain the PAS plasma concentrations above the MIC. In both doses the PAS plasma concentrations were well maintained throughout the 12-hour interval, with the once-daily dose's C_{12} and C_{max} being significantly higher (more than double the concentration).

The C_{12} was the last sample (C_{last}) of the twice-daily regimen (before the next dose), while the C_{last} of the once-daily regimen was the C_{24}.

In theory the difference between the pre-doses, C_0 and the C_{last}, is relatively small. However, in the study wide inter- and intraindividual variation in the concentrations was observed. As mention above, several factors can contribute to these variations, including pre-sample day dosing. In the study it was preferred to use the 24-hour sample concentrations since these samples were taken exactly 24 hours after the previous dose.

In the once- and twice-daily GSR-PAS study done by Peloquin[81], no PAS concentrations were detected in the 24-hour samples for the once-daily regimen. In contrast to their findings, PAS plasma concentrations in more than half of our study subjects were maintained above the MIC at the 24-hour sample. The remaining subjects were just below the MIC with some exceptions. It is however, important to note that Peloquin used a single 4 g dose, while an 8 g dose was used in this study.

Since the mean C_{12} for the once-daily was relatively high and PAS was still present in the C_{24} samples of those subjects with concentrations below the MIC, the once-daily dose probably maintained the PAS plasma concentrations above the MIC for the majority of the time between 12 and 24 hours.

With the simulated data of 1000 subjects (Table 18), predictions were made that the C_{12} and C_{24} of the simulated subjects that are using the once-daily regimen will have a 90% CI of (6.1, 81.2) and (0.06, 37.7), respectively. This indicates that most subjects' 24-hour concentration will fall below the MIC. However, the results were based on the first model with weight as the only covariate (Table 17).

Drug-drug interactions

Although only a few significant differences were found between the HIV positive and negative subjects, a significant difference was seen between their concentration profiles and statistics. The PAS plasma concentration and AUC_{12} of the HIV positive subjects was significantly lower than the HIV negative subjects. This observation can be associated with the disease state as well as the concomitant ARVs taken by the HIV positive subjects. The CYP2B6 substrate, EFV was identified as a covariate that increases the clearance of PAS by 45%. An interaction between PAS and EFV has so far not been reported in the literature.

EFV is predominantly metabolised by the CYP2B6, but is also a substrate for the CYP2A6, CYP3A4 and UGT2B7 enzymes [106]. As mentioned, a drug such as EFV can decrease or increase the clearance of other drugs by inhibiting or inducing the enzyme activity affecting those drugs. EFV is known to influence the enzyme activity of CYP3A4, CYP2B6, CYP2C9, CYP2C19, UGT2B7 and UGT 3A4 UGT [107,108,109]. Since several drugs are substrates for the CYP group, especially the CYP3A4, the drug interactions for EFV have been studied extensively.

PAS is not a substrate for any of the CYP enzymes; therefore, it is highly unlikely that the low PAS concentration is a result of induction of any of the CYP enzymes. Although PAS is predominantly acetylated by NAT1, it can also be glucuronidated by UGT1A9. As mentioned above EFV has an inducing and inhibiting effect on the UGT enzymes, including UGT1A9 [109]. Our observation could, therefore be a result of an inducing effect of EFV on the glucuronidation of PAS.

However, it is important to note that lamivudine and stavudine were also co-administered with EFV. During the screening for covariates, the concomitant medications were categorised according to their effect on the CYP enzymes, therefore the drugs that are metabolised by other enzymes were not screened.

Stavudine and lamivudine are classified as nucleoside reverse transcriptase inhibitors (NRTIs) and are predominately phosphorylated to their active metabolites. According to Coyne[14] interactions between the NRTIs and PAS are highly unlikely.

Since only a few HIV positive subjects were prescribed a regimen without EFV, it is difficult to conclude if the clearance is increased only by EFV or all the other ARVs. However, the increase in PAS clearance is not due to the disease state (HIV), since some HIV positive subjects that were not compliant to their ART had higher PAS concentrations.

The discovery of the effect of EFV on PAS clearance provided a new perspective and approach to the pharmacokinetic modelling. The 90% CI for the subjects taking EFV was much lower than those not taking EFV, for instance the C_{24} (once daily) of those taking EFV (Table 21) has a 90% CI of (0.89, 21.9), while those not taking EFV had a 90% CI of (1.4, 37.3) (Table 20). This indicates that most of the subjects receiving EFV with a single 8 g GSR-PAS dose will fall below the MIC, while fewer subjects will have concentrations below the MIC if they are not taking EFV.

In the simulated data (Table 21) for the subjects taking EFV (once-daily), the C_{12} 90% CI was (8.6, 58.1). Those not taking EFV had a 90% CI of (12.8, 89.7). This indicates that more subjects who are taking the EFV will have concentrations below the MIC between 12 and 24 hours than those not taking EFV.

Genetic variants and gene-gene interactions

Although no significant difference was found between the genotypes, the mean PK profiles of the different genotypes (Figure 28) indicate that *NAT1*4/1*4* (wild type), *NAT1*4/NAT1*27, NAT1*4/1*10* and *NAT1*10/*10* are fast acetylators. The mean PAS PK profiles of the two subjects with the *NAT1*4/NAT1*14A* genotype were much higher than the other genotypes, indicating that these subjects are slow acetylators. The results of the profiles are consistent with the literature [20,100].

With the grouping, the *NAT1*4* heterozygous and *NAT1*10* homozygous individuals (*NAT1*4/1*10, NAT1*4/1*14A* and *NAT1*10/1*10*) had an approximate 20% decrease in PAS clearance, compared to the wild type (*NAT 1*4* homozygous). This decrease may be due to the higher concentrations of the *NAT1*14A* and not the *NAT1*10* alleles. However, the *NAT1*10* alleles also have higher PAS concentrations than the wild type (Figure 28)

Although a relatively slower clearance was seen with the *NAT1*4* and *NAT1*10* heterozygous and fast-intermediate *NAT2* phenotype, no statistical significance was found.

Due to the small sample size, these results cannot conclusively indicate that there is a *NAT1* effect on the PAS disposition.

Tolerability and Safety

The measurement of the tolerability depended on the subject's understanding of the VAS forms and their ability to recall. Although the scales were thoroughly explained on each occasion, potential misinterpretation could still have occurred. For this reason, a short interview was conducted before the subjects completed the VAS scales. The interview provided a verification of the tolerability scores of the subjects.

There was a wide variation in the intolerability, but no significant differences were found between the two regimens. However, there were some noteworthy differences. According to the VAS scores, the subjects had more abdominal pain and cramps with the twice-daily regimen, while the subjects had more diarrhoea with the once-daily regimen. Diarrhoea is one of the symptoms related to gastrointestinal intolerance which the GSR-PAS formulation could not decrease [110].

It is important to note that most of the concomitant drugs prescribed to the subjects such as ethionamide, moxifloxacin, dapsone, ethambutol, INH and kanamycin, are also known to cause gastrointestinal intolerance [110]. The subjects were asked whether they had nausea before or after the administration of the concomitant drugs. The majority of the subjects indicated that they had nausea after receiving the concomitant drugs. The nausea could therefore either be due to the concomitant drugs or be a combined effect of PAS and the other drugs.

Intolerance is often caused by high drug plasma concentrations and since no distinctive difference could be found between the two regimens, the association between the C_{max} and gastrointestinal intolerance was investigated (Table 24). Diarrhoea and abdominal pain and cramps were associated with low concentrations, while nausea, vomiting and bloatedness were associated with higher concentrations. However, lower scores for the gastrointestinal intolerance (except vomiting) were given by the subjects with concentrations above 100 µg/ml. These results indicate that PAS adverse events are not necessarily related to the higher PAS plasma concentrations seen with the once-daily regimen.

In the post-study interview, more subjects claimed that the once-daily regimen was worse than the twice-daily regimen, which indicated that the subjects misinterpreted the scales or did not complete it with their full intent. However, there was consistency between the daily interview and VAS scoring.

Apart from gastrointestinal intolerance, other side effects observed were tiredness and loss of appetite. PAS reduces the thyroxin synthesis, which can be related to the tiredness. The drug may also cause drug-induced hepatitis which is also related to lack of energy/fatigue and the development of anorexia. Since the drug-induced hepatitis has symptoms (nausea, loss of appetite, diarrhoea and nausea) that are the same as the side effects of PAS, it was difficult to differentiate between the two. Therefore, rashes and fevers were used as a guide. However, no rashes or fevers were reported.

Clinical usefulness and considerations

In the comparison between the two regimens, the twice-daily regimen seems to be the preferable dose, as it is well tolerated and results in PAS concentrations well

above the MIC. However, since the once-daily regimen has proven that it can sustain PAS plasma concentrations above the MIC or for the majority of time after 12 hours, the once-daily dosing may be useful in some cases.

Since the PAS concentrations and AUC_{12} of the HIV positive subjects receiving ARVs such as EFV were significantly lower, the once-daily GSR-PAS regimen may be less suitable in these patients. The patients co-administrating ARVs and PAS need to be encouraged to be compliant to the PAS and ART to avoid developing drug resistance.

Although PAS is a bacteriostatic agent, its potential to have an early bactericidal activity (EBA) with a single high dose of 15 g has been evaluated by Jindani et al.[103]. Even though the EBA 2-14 was low at 0.076, a moderate EBA 0-2 of 0.259 was reported for PAS. Unfortunately Jindani et al[103] did not measure PAS plasma concentrations after a single 15 g dose, but according to Walter and Heilmeyer [111] a 15-17 g dose can produce concentrations between 100-200 µg/ml.

The single high GSR-PAS dose used in the present study produced high concentrations that were in some cases above 100 µg/ml. However, EBA tests were not preformed, therefore the study was not able to confirm if the high concentrations have any early bactericidal effects. If it is possible to confirm that a concentration more than 100 µg/ml will result in an early bactericidal affect, a higher GSR-PAS dose could provide a combination of a bacteriostatic and bactericidal activity. More clinical data is required to confirm if once-daily dosing will be effective.

Jindani et al[103] studied PAS as monotherapy, while in this study various other concomitant drugs were used, including a high dose of INH, which may be beneficial or harmful (i.e. neurotoxicity a result of increase INH levels). In addition, most of the concomitant drugs and PAS are metabolised by the liver and excreted by the kidneys, therefore increasing the risk for hepatotoxicity and renal failure. The two subjects, who developed renal impairment after the study were on capreomycin (known to cause renal failure) for a long period, thus the study cannot conclude that the impairment was caused by the high GSR-PAS dose. Prospective studies are needed to determine the safety of the once-daily dose for a longer period than in the present study.

Study limitations and future work

Design

Although several interesting and clinically important outcomes were provided by the study, there were some limitations. It would have been ideal to include a washout period for the crossover design. This was unfortunately not possible, since the subjects were receiving the investigated drugs as part of their treatment regimen. It will be worth doing parallel trials or a 3-4 week cross-over trial, with a week in between the once- and twice-daily regimens. The extra week will then serve as the washout period and will assist in determining the intolerance of the different regimens.

The two week PK study was only able to determine the short term effect of the once-daily regimen; therefore an extensive study is needed to assess the safety and efficacy of the 8 g dose over a longer period. Addition clinical tests and culturing will be required for these assessments.

Clinical approaches

The subjects complained of not having an appetite in the morning after taking the single 8 g dose, therefore a future investigation could assess administration of PAS several hours after breakfast. Later administration may not only increase the tolerability, but may increase the PAS absorption, since PAS absorption is increased by food intake [40].

Although the VAS form is a standard approach to determine tolerability, more methods need to be considered in prospective studies. Interviews or observations could be more feasible than VAS, but with a few adjustments to the VAS it could still be a valuable tool.

Furthermore, other side effects need to be determined for the single daily dose. Interviews and physical examinations will be a good approach to determine the other side effects.

Only a small group of subjects participated in the study, and therefore future work is needed to determine the safety and tolerability of the once-daily regimen, especially when prescribed in combination with other drugs that have the same toxic effect and

those that can reach toxic levels due to PAS interactions, such as INH.

Drug-drug interactions

The interactions between the ARVs and PAS need to be confirmed in a larger study, where both drugs are analysed to determine if PAS also has an effect on the ARVs. Prospective work will be to analyse other concomitant drugs with PAS and investigate potential interactions of PAS with the older and newer TB and ARV drugs.

Genetic factors

In order to describe all the different genetic variants and phenotypic activity in the population, a larger sample size is required. A feasible approach to genetics will be to analyse the other genes that may be directly or indirectly involved in the ADME of PAS. The investigation of genetic factors affecting the metabolism of concomitant drugs such as EFV and INH will be a valuable approach to predict the clearance of PAS and development of toxicity.

Minimum Bactericidal Concentration

Furthermore, *in vitro* and *in vivo* bactericidal testing is required to determine if PAS has early bactericidal activities with high concentrations.

Pharmacometric evaluations

A next step in the pharmacometric evaluation of the outcome of the present study could be the development of pharmacokinetic and pharmacodynamic models that correlate the relationship between the subjects' characteristics, PAS plasma concentrations, other drugs, *M. tuberculosis* cultures and tolerability.

Chapter Seven: Conclusion

The study provided information about the pharmacokinetics of PAS in adults with M/XDR-TB. It showed that a single 8 g dose of GSR-PAS leads to significantly higher PAS plasma concentrations than a 4 g dose given twice-daily. This higher dose is able to sustain the PAS plasma concentrations above the MIC of 1 µg/ml throughout the dosing interval of 24 hours in a high proportion of non-HIV infected adult patients with M/XDR-TB. A single dose can therefore not only provide a prolonged bacteriostatic effect, but possibly increase the bactericidal effect of GSR-PAS.

Therefore, the 8 g once-daily dose can be considered for future regimens when outpatient-based, supervised treatment is needed. Both regimens are reasonably well tolerated on the background of a cocktail of accompanying anti-tuberculosis agents. Due to lower exposure the once-daily regimen will be less suitable for HIV-infected subjects on ARV-drugs. PAS and ARV interactions need to be clarified before the 8 g once-daily dose can be recommended for the HIV co-infected patients on ART.

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Appendices

Appendix A: Package Insert of Granules delayed-release PAS (PASER)

PASER - aminosalicylic acid granule, delayed release

Jacobus Pharmaceutical Company, Inc.

4 grams

Caution: Federal, law prohibits dispensing without prescription.

JACOBUS PHARMACEUTICAL COMPANY, INC.

Princeton, NJ 08540

2A JULY, 1996

PASER® GRANULES

(aminosalicylic acid granules)

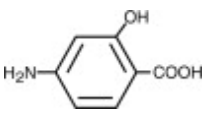
DESCRIPTION

PASER granules are a delayed release granule preparation of aminosalicylic acid (p-aminosalicylic acid; 4-aminosalicylic acid) for use with other anti-tuberculosis drugs for the treatment of all forms of active tuberculosis due to susceptible strains of tubercle bacilli. The granules are designed for gradual release to avoid high peak levels not useful (and perhaps toxic) with bacteriostatic drugs.

Aminosalicylic acid is rapidly degraded in acid media; the protective acid-resistant outer coating is rapidly dissolved in neutral media so a mildly acidic food such as orange, apple or tomato juice, yogurt or apple sauce should be used.

Aminosalicylic acid (p-aminosalicylic acid) is 4-Amino-2-hydroxybenzoic acid. PASER granules are the free base of aminosalicylic acid and do NOT contain sodium or a sugar. The molecular formula is $C_7H_7NO_3$ with a molecular weight of 153.14. With heat p-aminosalicylic acid is decarboxylated to produce CO_2 and m-aminophenol. If the airtight packets are swollen, storage has been improper. DO NOT USE if packets are swollen or the granules have lost their tan color and are dark brown or purple.

The structural formula is:



PASER granules are supplied as off-white tan colored granules with an average diameter of 1.5 mm and an average content of 60% aminosalicylic acid by weight. The acid resistant outer coating will be completely removed by a few minutes at a neutral pH. The inert ingredients are:

colloidal silicon dioxide

dibutyl sebacate

hydroxypropyl methyl cellulose

methacrylic acid copolymer

microcrystalline cellulose

talc

The packets contain 4 grams of aminosalicylic acid for oral administration three times a day by sprinkling on apple sauce or yogurt to be eaten without chewing. Suspension in an acidic fruit drink such as orange juice or tomato juice will protect the coating for at least 2 hours. Swirling the juice in the glass will help resuspend the granules if they sink.

CLINICAL PHARMACOLOGY

Mechanism of Action: Aminosalicylic acid is bacteriostatic against Mycobacterium tuberculosis. It inhibits the onset of bacterial resistance to streptomycin and isoniazid. The mechanism of action has been postulated to be inhibition of folic acid synthesis (but without potentiation with antifolic compounds) and/or inhibition of synthesis of the cell wall component, mycobactin, thus reducing iron uptake by M. tuberculosis.

Characteristics: The two major considerations in the clinical pharmacology of aminosalicylic acid are the prompt production of a toxic inactive metabolite under acid conditions and the short serum half-life of one hour for the free drug. Both are

discussed below. After two hours in simulated gastric fluid, 10% of unprotected aminosalicylic acid is decarboxylated to form meta-aminophenol, a known hepatotoxin. The acid-resistant coating of the PASER granules protects against degradation in the stomach. The small granules are designed to escape the usual restriction on gastric emptying of large particles. Under neutral conditions such as are found in the small intestine or in neutral foods, the acid-resistant coating is dissolved within one minute. Care must be taken in the administration of these granules to protect the acid-resistant coating by maintaining the granules in an acidic food during dosage administration. Patients who have neutralized gastric acid with antacids will not need to protect the acid resistant coating with an acidic food since no acid is present to spoil the drug. Antacids may influence the absorption of other medications and are not necessary for PASER consumed with an acidic food.

Because PASER granules are protected by an enteric coating absorption does not commence until they leave the stomach; the soft skeletons of the granules remain and may be seen in the stool.

Absorption and excretion: In a single 4 gram pharmacokinetic study with food in normal volunteers the initial time to a 2 µg/ml serum level of aminosalicylic acid was 2 hours with a range of 45 minutes to 24 hours; the median time to peak was 6 hours with a range of 1.5 to 24 hours; the mean peak level was 20 µg/ml with a range of 9 to 35 µg/ml; a level of 2 µg/ml was maintained for an average of 7.9 hours with a range of 5 to 9; a level of 1 µg/ml was maintained for an average of 8.8 hours with a range of 6 to 11.5 hours. The recommended schedule is 4 grams every 8 hours. 80% of aminosalicylic acid is excreted in the urine, with 50% or more of the dosage excreted in acetylated form. The acetylation process is not genetically determined as is the case for isoniazid. Aminosalicylic acid is excreted by glomerular filtration; although previously reported otherwise, probenecid, a tubular blocking agent, does not enhance plasma concentration. In a 1954 study thyroxine synthesis but not iodide uptake was reported reduced about 40% when the sodium salt (not PASER granules) of aminosalicylic acid was administered one hour before radio-iodine; the sodium salt typically produces a serum level over 120 µg/ml at one hour lasting one hour. Occasional goiter development can be prevented by the administration of thyroxine but not iodide. Penetration into the cerebrospinal fluid occurs only if the meninges are inflamed. Approximately 50-60% of aminosalicylic acid is protein bound; binding is reported to be reduced 50% in kwashiorkor. Microbiology: The aminosalicylic acid MIC for M. tuberculosis in 7H11 agar was less than 1.0 µg/ml for

nine strains including three multidrug resistant strains, but 4 and 8 µg/ml for two other multidrug resistant strains. The 90% inhibition in 7H12 broth (Bactec) showed little dose response but was interpreted as being less than or equal to 0.12-0.25 µg/ml for eight strains of which three were multi-resistant, 0.50 µg/ml for one resistant strain, questionable for four non-resistant strains and greater than 1µg/ml for one non-resistant and three resistant strains. Aminosalicylic acid is not active in vitro against M. avium.

INDICATIONS AND USAGE

PASER is indicated for the treatment of tuberculosis in combination with other active agents. It is most commonly used in patients with Multi-drug Resistant TB (MDR-TB) or in situations when therapy with isoniazid and rifampin is not possible due to a combination of resistance and/or intolerance. When PASER is added to the treatment regimen in patients proven or suspected drug resistance, it should be accompanied by at least one and preferably two other new agents to which the patient's organism is known or expected to be susceptible.

CONTRAINDICATIONS

Hypersensitivity to any component of this medication.

Severe renal disease.

Patients with severe renal disease will accumulate aminosalicylic acid and its acetyl metabolite but will continue to acetylate, thus leading exclusively to the inactive acetylated form; deacetylation, if any, is not significant.

The half-life of free aminosalicylic acid in renal disease is 30.8 minutes in comparison to 26.4 minutes in normal volunteers, but the half-life of the inactive metabolite is 309 minutes in uremic patients in comparison to 51 minutes in normal volunteers. Although aminosalicylic acid passes dialysis membranes, the frequency of dialysis usually is not comparable to the half-life of 50 minutes for the free acid. Patients with end stage renal disease should not receive aminosalicylic acid.

WARNINGS

Liver Function

In one retrospective study of 7492 patients on rapidly absorbed aminosalicylic acid preparations, drug-induced hepatitis occurred in 38 patients (0.5%); in these 38 the first symptom usually appeared within three months of the start of therapy with a rash

as the most common event followed by fever and much less frequently by GI disturbances of anorexia, nausea or diarrhea. Only one patient was diagnosed on routine biochemistry.

Premonitory symptoms in 90% of these 38 patients preceded jaundice by a few days to several weeks with the mean time of onset 33 days with a range of 7-90 days. Half of the adverse reactions occurred during the third, fourth or fifth weeks. When aminosalicylic acid-induced hepatitis was diagnosed, hepatomegaly was invariably present with lymphadenopathy in 46%, leucocytosis in 79%, and eosinophilia in 55%. Prompt recognition with discontinuation led to the recovery of all 38 patients. If recognized in the premonitory stage, the reaction is reported to "settle" in 24 hours and no jaundice ensues. From other reported studies failure to recognize thereaction can result in a mortality of up to 21%. The patient must be monitored carefully during the first three months of therapy and treatment must be discontinued immediately at the first sign of a rash, fever or other premonitory signs of intolerance.

PRECAUTIONS

(1)General:

All drugs should be stopped at the first sign suggesting a hypersensitivity reaction. They may be restarted one at a time in very small but gradually increasing doses to determine whether the manifestations are drug-induced and, if so, which drug is responsible. Desensitization has been accomplished successfully in 15 of 17 patients starting with 10 mg aminosalicylic acid given as a single dose. The dosage is doubled every 2 days until reaching a total of 1 gram after which the dosage is divided to follow the regular schedule of administration. If a mild temperature rise or skin reaction develops, the increment is to be dropped back one level or the progression held for one cycle. Reactions are rare after a total dosage of 1.5 grams.

Patients with hepatic disease may not tolerate aminosalicylic acid as well as normal patients, even though the metabolism in patients with hepatic disease has been reported to be comparable to that in normal volunteers.

(2) Information for Patients:

The patient should be advised that the first signs of hypersensitivity include a rash, often followed by fever, and much less frequently, GI disturbances of anorexia,

nausea or diarrhea. If such symptoms develop, the patient should immediately cease taking the medication and arrange for a prompt clinical visit. Patients should be advised that poor compliance in taking anti-TB medication often leads to treatment failure, and, not infrequently, to the development of resistance of the organisms in the individual patient.Patients should be advised that the skeleton of the granules may be seen in the stool.

The coating to protect the PASER granules dissolves promptly under neutral conditions; the granules therefore should be administered by sprinkling on acidic foods such as apple sauce or yogurt or by suspension in a fruit drink which will protect the coating, but the granules sink and will have to be swirled. The coating will last at least 2 hours in either system. All juices tested to date have been satisfactory; tested are: tomato, orange, grapefruit, grape, cranberry, apple, “fruit punch”.

Patients should be advised to store PASER in a refrigerator or freezer. PASER packets may be stored at room temperature for short periods of time.

Patients should be advised NOT to use if the packets are swollen or the granules have lost their tan color and are dark brown or purple. The patient should inform the pharmacist or physician immediately and return the medication.

3) Laboratory Tests:

Aminosalicylic acid has been reported to interfere technically with the serum determinations of albumin by dye-binding, SGOT by the azoene dye method and with qualitative urine tests for ketones, bilirubin, urobilinogen or porphobilinogen.

(4) Drug Interactions:

Aminosalicylic acid at a dosage of 12 grams in a rapidly available form has been reported to produce a 20 percent reduction in the acetylation of isoniazid, especially in patients who are rapid acetylators; INH serum levels, half-lives and excretions in fast acetylators still remain half of the levels seen in slow acetylators with or without p-aminosalicylic acid. The effect is dose related and, while it has not been studied with the current delayed release preparation, the lower serum levels with this preparation will result in a reduced effect on the acetylation of INH.

Aminosalicylic acid has previously been reported to block the absorption of rifampin. A subsequent report has shown that this blockade was due to an excipient not

included in PASER granules. Oral administration of a solution containing both aminosalicylic acid and rifampin showed full absorption of each product.

As a result of competition, Vitamin B12 absorption has been reduced 55% by 5 grams of aminosalicylic acid with clinically significant erythrocyte abnormalities developing after depletion; patients on therapy of more than one month should be considered for maintenance B12.
A malabsorption syndrome can develop in patients on aminosalicylic acid but is usually not complete. The complete syndrome includes steatorrhea, an abnormal small bowel pattern on x-ray, villus atrophy, depressed cholesterol, reduced D-xylose and ironabsorption. Triglyceride absorption always is normal. In one literature report 8 hours after the last dosage of aminosalicylic acid at 2 gm qid serum digoxin levels were reduced 40% in two of ten patients but not changed in the remaining eight.

(5) Carcinogenesis, mutagenesis, impairment of fertility:

Sodium aminosalicylate produced an occipital bone defect, probably with a dose response, when administered to ten pregnant Wistar rats at five doses from 3.85 to 385 mg/kg from days 6 to 14. There were no significant changes from controls in any group in corpora lutea, early resorptions, total resorptions, fetal death, litter size, or hematomas. For all except the 77 mg/kg group, fetal weights were significantly greater than controls. Chinchilla rabbits on 5 mg/kg from days 7 to 14 did not show any significant differences as compared to controls for the same parameters studied. Sodium aminosalicylic acid was not mutagenic in Ames tester strain TA 100. In human lymphocyte cultures in-vitro clastogenic effects of achromatic, chromatid, isochromatic breaks or chromatid translocations were not seen at 153 or 600 µg/ml. At 1500 and 3000 µg/ml there was a dose related increase in chromatid aberrations.
Patients on isoniazid and aminosalicylic acid have been reported to have an increased number of chromosomal aberrations as compared to controls.

(6) Pregnancy: Pregnancy Category C:

Aminosalicylic acid has been reported to produce occipital malformations in rats when given at doses within the human dose range.Although there probably is a dose response, the frequency of abnormalities was comparable to controls at the highest level tested (two times the human dosage). When administered to rabbits at 5 mg/kg,

throughout all three trimesters, no teratologic or embryocidal effects were seen. Literature reports on aminosalicylic acid in pregnant women always report coadministration of other medications.

Because there are no adequate and well controlled studies of aminosalicylic acid in humans, PASER granules should be given to a pregnant woman only if clearly needed.

(8) Nursing mothers:

After administration of a different preparation of aminosalicylic acid to one patient, the maximum concentration in the milk was 1 □g/ ml at 3 hours with a half-life of 2.5 hours; the maximum maternal plasma concentration was 70 µg/ml at two hours.

ADVERSE EFFECTS

The most common side effect is gastrointestinal intolerance manifested by nausea, vomiting, diarrhea, and abdominal pain. Hypersensitivity reactions: Fever, skin eruptions of various types, including exfoliative dermatitis, infectious mononucleosis-like, or lymphoma-like syndrome, leucopenia, agranulocytosis, thrombocytopenia, Coombs' positive hemolytic anemia, jaundice, hepatitis, pericarditis, hypoglycemia, optic neuritis, encephalopathy, Leoffler's syndrome, vasculitis and a reduction in prothrombin. Crystalluria may be prevented by the maintenance of urine at a neutral or an alkaline pH.

OVERDOSAGE

Overdosage has not been reported.

DOSAGE AND ADMINISTRATION

PASER granules should be administered with other drugs to which the organism is known or expected to be susceptible. It is most commonly administered to patients with Multi-drug Resistant TB (MDR-TB) or in other situations in which therapy with isoniazid or rifampin is not possible due to a combination of resistance and/or intolerance. The adult dosage of four grams (one packet) three times per day or correspondingly smaller doses in children should be given by sprinkling on apple sauce or yogurt or by swirling in the glass to suspend the granules in an acidic drink

such as tomato or orange juice.
DO NOT USE if packet is swollen or the granules have lost their tan color, turning dark brown or purple.

HOW SUPPLIED

Carton of 30 PASER packets (NDC 49938-107-04).
Each packet contains four grams aminosalicylic acid.
PASER granules are supplied in packets containing 4 grams of aminosalicylic acid for administration three times a day by suspension in an acidic drink or food with a pH less than 5. Examples include apple sauce, yogurt, tomato or orange juice.
Distributors and Pharmacists: Store below 59°F (15°C) (in a refrigerator or freezer). Patients are urged to store PASER in a refrigerator or freezer. PASER packets may be stored at room temperature for short periods of
time. AVOID EXCESSIVE HEAT. DO NOT USE if packet is swollen or the granules have lost their tan color, turning dark brown or purple.

JACOBUS PHARMACEUTICAL CO. INC.
P.O. Box 5290
Princeton, NJ 08540
2A JULY, 1996

PRINCIPAL DISPLAY PANEL

Principal Display Panel – 4g Carton Label

NDC 49938-107-04
PASER® Aminosalicylic Acid
Delayed-release Granules
4 Grams
30 Unit Dose Packets
STORE BELOW 59°F (15°C)
ALTERNATIVELY STORE IN A
REFRIGERATOR OR FREEZER
AVOID EXCESSIVE HEAT

Caution: Federal law prohibits dispensing without prescription

Appendix B: Protocol of Study 1

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Clinical Pharmacology of Second-line Antituberculosis Drugs.

RESEARCH PROTOCOL

Investigators:

Anthony Liwa, MD, BSc (Hons) [1]
Bernd Rosenkranz, MD, PhD, FFPM[1]
Patrick Bouic, PhD[2]
Simon Schaaf, MBChB[3]
Peter Donald, MBChB[3]

Division of Pharmacology (1)
Synexa Group, South Africa (2)
Department of Paediatrics (3)

Address of correspondence:
Prof. Bernd Rosenkranz
Professor and Head
Division of Pharmacology

[Faculty of Health Sciences, Department of Medicine, Division of Pharmacology, PO Box 19063, Tygerberg, 7505, Cape Town, South Africa

Department of Medicine
University of Stellenbosch
PO Box 19063
Tygerberg, Cape Town, 7505
South Africa
Phone: +27-21-938 9331
Mobile: +27-82-955 0017
Fax: +27-21-932 6958
Mail: Rosenkranz@sun.ac.za

Preface

In the current protocol we propose to study:

1. The *in vivo* pharmacokinetics (PK) of the second-line antituberculosis drugs terizidone and para aminosalycylic acid (PAS) in children

2. The pharmacokinetics (PK) of the second-line antituberculosis drug para-aminosalycylic acid (PAS) in adults

3. The pharmacogenetics of genes relevant for drug elimination such as N-acetyltransferase among patients treated with PAS for treatment of drug resistant tuberculosis.

4. The *in vitro* metabolism of the second-line anti tuberculosis drugs determination using liver microsomes.

The primary objective of the protocol is to evaluate the PK profile of terizidone and PAS in children by single dose study under strictly managed conditions following a defined protocol at set time points after dosing in children and adults. In parallel, we plan to assess the pharmacogenetics of PAS, the *in vitro* metabolic stablility and the effect of ethionamide, terizidone and PAS on different metabolizing enzymes by using standardized methodology.

Research protocol

Title: Pharmacokinetics and dosing rationale of terizidone and para-aminosalicylic acid (PAS) in children and the evaluation of the in vitro metabolism of ethionamide, terizidone and PAS.

Protocol synopsis

Tuberculosis continues to cause an unacceptably high toll of disease and death among children worldwide. Available data has suggested an exponential rise in the proportion of the tuberculosis case load caused by children as the incidence of tuberculosis rises so that children may constitute nearly 40 per cent of the case load in certain high incidence communities. Until now no trials have been done in children to establish optimum antituberculosis treatment regimens and current treatment guidelines are largely inferred from adult data. This is despite the fact that pharmacokinetics in children and especially very young children are likely to be different from that in adults. Furthermore resistance to frontline therapeutics has resulted in treatment of patients with “second line“ agents that are considered more toxic, and the experience of these drugs with children is even more limited. Tuberculosis management in children therefore presents particularly difficult challenges, and advances in paediatric tuberculosis research could provide insights and opportunities for tuberculosis control.

In the proposed protocol we aim to conduct the pharmacokinetic study of second line anti tuberculosis drugs terizidone and PAS in childhood TB across the range of dosages and ages in order to determine the validity of the currently suggested dose based on adult data.

We will also conduct the in vitro study of the susceptibility and stability of the second line anti TB drugs (ETH, terizidone and PAS) to biotransformation when subjected to metabolic enzymes. These drugs will also be incubated with the substrates of major enzymes/isoenzymes i.e. cytochrome P450 enzymes (CYPs), flavin containing monooxygenases (FMO) and uridine glucoronosyl transferases (UGT) to determine whether they have any effects on these enzymes.

Research hypothesis

1. Because of developmental changes, the pharmacokinetics of terizidone and PAS and resulting serum levels may differ in children of different age groups.

2. Young children require a higher body weight adjusted dosage of the "second-line" antituberculosis agents, terizidone and PAS than adults to achieve equivalent serum concentrations.

3. Because of the genetic variations of the metabolizing enzymes (*N*-acetyltransferase) the serum concentrations may differ in different individuals.

4. The drugs under investigation are metabolically unstable and we therefore will investigate their in vitro metabolism and potential for drug-drug interactions.

Aim of this study

1. To document the descriptive pharmacokinetics of the second-line antituberculosis agents terizidone and PAS in children, in an attempt to provide an insight to the dosing rationale of these compounds; these data will be compared to the results obtained in adult patients as a reference group.
2. To investigate the genetic variation of NAT1and NAT2 in patients with tuberculosis treated by PAS.
3. To evaluate the stability and susceptibility to biotransformation of the second-line antituberculosis drugs (ETH, terizidone and PAS) when subjected to metabolic enzymes
4. To generate baseline data and methodology for the future in vitro metabolic analysis of new compounds at the Division of Pharmacology.

Study methodology

The proposed study is a prospective longitudinal clinical as well as laboratory study; We plan to recruit children 13 years or younger and a group of adults 18 years or older with probable or confirmed drug resistant tuberculosis admitted to Brooklyn Hospital for Chest Diseases (BHCD) and In this explorative study, up to 40 children (for both drugs) and up to 40 adults will be enrolled.

We plan to measure the serum concentrations at different set points after dosing in different age groups. The methodology requires 1 ml of blood for each assay and blood will be drawn during each assessment via an indwelling catheter that will be inserted on the morning of the evaluation. The indwelling catheter will be used to minimize the number of venipunctures required throughout the investigation. The serum concentrations will be determined by an established high performance liquid chromatography/mass spectrometry method (HPLC-MS).

Methodology for the *in-vitro* study will involve the incubation of the test compound at concentrations with the enzyme source (Human liver microsomes (HLM)), and S9 fractions) with appropriate positive and negative controls. The time spacing and replicate number will be according to standardized procedures. The amount of parent drug remaining in the incubation media will be analyzed by HPLC/MS detection. The current procedure will also involve the incubation of substrates for specific isoenzymes with the test compounds using HLM to determine the effects of these drugs on different metabolizing enzymes.

Microsoft excel will be used to capture the data and STATISTICA version 8 (StatSoft Inc. (2008) STATISTICA (data analysis software system) will be used to analyse the data. Summary statistics will be used to describe the variables. Distribution of variables will be presented as histograms and or as frequency tables. Medians or means will be used as the measures of central location for ordinal and continuous responses and standard deviations and quartiles as indicators of spread.

Time plan

We have planned to submit the protocol between August-September 2009 and have full Ethics approval for the study to start shortly thereafter. The aim is to collect data according to the protocol within a year. Data analysis and writing of the manuscript could be completed in 2 months. It would be possible to aim at the final manuscript at the end of year 2010 for the final master's thesis presentation and graduation. At the time of the submission of this amendment, the clinical project is ongoing (paediatric population).

The in vitro study will have a specific timeline, its implementation will commence after the approval from the ethical committee and the arrival of the required study materials. At the time of submission of this amendment, the laboratory work is being initiated.

Conclusion

The data generated in this study will be used as guidance for future studies involving much larger populations. We plan to present the data at congresses and to write this up for publications.

Funding and budget

Partial funding from the departmental funds is readily available. In addition, funding is

available from NRF grants provided to Prof. P. Donald.

Introduction

Tuberculosis continues to cause an unacceptably high toll of disease and death among children worldwide. (**Newton et al., 2008**) Approximately 8.3million new TB cases were diagnosed globally in 2000. It is estimated that 884,019 (11%) cases occurred among children, with the highest burden noted in areas of endemicity. A survey conducted in Cape Town, South Africa, indicated that 13% of cases entered into the register were found among children < 13 years of age. (**Marais et al., 2004**) Children are particularly vulnerable to severe disease and death following infection. Few trials have been done in children to establish optimum antituberculosis treatment regimens and current treatment guidelines are largely inferred from adult data. (**Newton et al., 2008)** Resistance to frontline therapeutics, most notably, isoniazid (INH) and rifampicin, results in treatment of patients with “second-line” agents that are less effective and/or more toxic. Among the second tier drugs for the treatment of multidrug-resistant tuberculosis, ethionamide (ETH**), (Vanneli et al., 2002)** and terizidone or cycloserine (CS) are generally used first because of efficacy, side effect profile, and historical use in tuberculosis **(Murkherjee et al., 2004**). Dosages for children are based on weight and extrapolated from data from studies with adults, yet pharmacokinetics for children, especially very young children, is likely to be different than for adults. Studies of ethambutol and pyrazinamide have found lower plasma drug levels and shorter half-lives in children than in adults using the same dosages. **(Graham et al., 2006)** Similar conclusions were drawn from a study of isoniazid pharmacokinetics in South African children. (**Schaaf et al., 2005)**

In the light of the above remarks we conclude that tuberculosis management in children presents particularly difficult challenges, and that advances in paediatric tuberculosis research could provide wider insights and opportunities for tuberculosis control.

In the proposed protocol we aim to study the pharmacokinetics of second-line anti tuberculosis drugs terizidone and PAS in childhood TB across the range of dosages and ages in order to determine the validity of the currently suggested doses based on adult target concentrations as well as pharmacokinetic data obtained in a reference group of adult patients in the present study..

We also aim to conduct the in vitro evaluation of susceptibility and stability of the

second line anti TB drugs (ETH, terizidone and PAS) to biotransformation when subjected to metabolic enzymes. These drugs will also be incubated with the substrates of major enzymes/isoenzymes i.e. cytochrome P450 enzymes (CYPs), flavin containing monooxygenases (FMO) and uridine glucoronosyl transferases to determine whether they have any inhibitory effects on these enzymes.

Background information and literature review

Burden of TB

Tuberculosis (TB) is a highly contagious chronic granulomatous bacterial infection, and is still a leading killer worldwide. TB has returned with a new face and the global scourge of multi-drug resistant TB (MDR TB) is reaching epidemic proportions. Rates of HIV infection among TB patients are correspondingly high, exceeding 60% in South Africa, Botswana, Zambia, and Zimbabwe. **(Du Toit et al., 2006)** It is estimated that one third of the world's population is infected with *mycobacterium tuberculosis*, and that each year, about 9 million people develop TB, of whom about 2 million die. Of the 9 million annual TB cases, about 1 million (11%) occur in children. Of these childhood cases, 75% occur annually in 22 high burden countries. (**WHO Guidelines., 2006)** TB in the Western Cape Province was 520/100,000 in 1998 and 678/100,000 in 2003**. (Cape Town TB Control Progress report 1997-2003)**

Tuberculosis in children

Children are mainly infected by adult pulmonary TB source cases and childhood TB therefore reflects the intensity of ongoing transmission of *Mycobacterium tuberculosis* within a community. (**Schaaf et al., 2005)** Since most children acquire the organisms from adults in their surroundings, the epidemiology of childhood tuberculosis follows that in adults. Tuberculosis case load represented by children suggest an exponential rise in the proportion of the tuberculosis case load caused by children as the tuberculosis rises, nearly 40 per cent of the case load in certain high incidence communities. **(Donald PR., 2002)** In Cape Town, South Africa, childrensuffer considerable morbidity: 207/439 (47.1%) had disease manifestations other than uncomplicated lymph node disease, while 26/439 (5.9%) were diagnosed with disseminated (miliary) disease and/or tuberculous meningitis (TBM). (**Marais et al., 2004)**

Pediatric pharmacology: Consequences of immaturity

Developmental changes are responsible for differences in drug disposition seen

throughout childhood, therefore the weight-adjusted drug dose may not be the same for different age groups. During a period of latent or rapid growth, some drugs that may cause severe or protracted toxicity can alter the final mature expression of a system.. These concepts of drug effects on host effect on drug need to be appreciated for appropriate use of drugs in children. **(Pradhan et al., 1986)** Age-related differences in PK, in addition to those of body size, can be used to guide calculations. Altered absorption, distribution, and elimination are most marked in the newborn, but for many drugs, disposition process may equal or exceed the adult capacity by late infancy and /or childhood. **(Pradhan et al., 1986)**

Drug-resistant Tuberculosis

The treatment and control of TB is more complex when the organism is resistant to the action of antituberculosis drugs. MDR-TB are associated with mortality as high as 43–93% in adults, despite limited information in children, resistance patterns in children have generally been found to be similar to those of adults from the same areas and similar backgrounds. **(Nelson et al., 2004)** Traditionally, patients with drug-resistant tuberculosis are classified as having acquired or primary drug resistance on the basis of a history of previous treatment. In the survey in 35 countries, the median prevalence of primary resistance to any antituberculous drug was 9·9% (range 2-41) and that of acquired resistance was 36%. **(Van Rie et al,. 2000**). In a study conducted at Tygerberg Children Hospital (TCH). Among 313 children (range 2 weeks -/12.9 years) in whom drug susceptibility test (DST) results were available, 40 (12.8%) and 17 (5.4%) were infected with strains resistant to isoniazid and rifampicin, respectively. All who had rifampicin-resistant strains had co-existent resistance to isoniazid (MDR TB). Only 1/40 (2.5%) children with resistance to isoniazid and/or rifampicin was also resistant to ethambutol. **(Schaaf et al., 2007)**
Extensively drug resistant tuberculosis has now been reported from 45 countries, though this almost certainly underestimates its true extent as many countries lack laboratory facilities to detect resistance to second line drugs. The outbreak in South Africa was particularly alarming because, most patients with extensively drug resistant tuberculosis had no history of tuberculosis treatment, implying person to person transmission of extensively drug resistant tuberculosis, and because of evidence of transmission in healthcare settings. (**Grant et al., 2008)**

Second-line anti-tuberculosis drugs

Terizidone

Terizidone (a derivative of cycloserine) It has a bacteriostatic effect at the usual dose, is used in combination with other second-line agents in the treatment of MDR-TB, as it does not share cross resistance with other anti TB drugs. It is believed to have lower incidence of side effects than cycloserine. **(WHO, Geneva., 2002. SAMF 8th Ed, 2008)**

Para-aminosalicylic acid (PAS)

PAS is bacteriostatic drug, *in-vitro*, most strains of the mycobacterium tuberculosis are sensitive to a concentration of 1µg/ml. It is administered orally in a daily dose of 10 to 12g. **(Goodman and Gilman., 2006)** PAS is used primarily as a second-line drug to treat MDR TB. **(Mathys et al., 2009)**

Hypothesis

1. Because of developmental changes, the pharmacokinetics of terizidone and PAS and resulting serum levels may differ in children of different age groups.
2. Young children require a higher body weight adjusted dosage of the "second-line" antituberculosis agents, terizidone and PAS than adults to achieve equivalent serum concentrations.
3. Because of the genetic variations of the metabolizing enzyme (*N*-acetyltransferase) the serum concentrations may differ in different individuals.
4. The drugs under investigation are metabolically unstable and have a potential for drug-drug interactions.

Rationale

Children in endemic settings are highly susceptible to TB infection and disease. Because of the rising rates of MDR TB and because children are at a higher risk of developing disseminated forms of tuberculosis, information on drug dosing for second line drugs in this patient population is urgently needed.

Aim of study

1. To document the descriptive pharmacokinetics of the second-line antituberculosis agents terizidone and PAS in children, in an attempt to

provide an insight to the dosing rationale of these compounds; these data will be compared to the results obtained in adult patients as a reference group.

2. Investigate the genetic variation of NAT1and NAT2 in patients with tuberculosis treated by PAS.

3. To evaluate the susceptibility and stability of the second line anti TB drugs (ETH, terizidone and PAS) to biotransformation when subjected to metabolic enzymes.

4. To generate baseline data and methodology for the future applicationif *in-vitro* metabolism studies at the Division of Pharmacology.

Study methodology

Study design

1. A prospective, longitudinal, hospital based clinical pharmacokinetic study.

2. An *in-vitro* laboratory based analytical study

Study setting and study population

The study recruitment sites will be Tygerberg Children's Hospital (TCH) and Brooklyn Chest Hospital (BCH) both associated with the Stellenbosch University and situated in the district of Cape Town. **TCH** is a tertiary level hospital and serves as referral hospital for complicated TB cases from the surrounding high-burden communities.
BHC is a specialized TB hospital, focusing on the long-term treatment of complicated and drug-resistant TB.

Subjects and sites

Children with probable or confirmed drug-resistant tuberculosis admitted to the above mentioned hospitals, who are on treatment with **terizidone** and/or **PAS**.

Sample size considerations

In this explorative study a valid estimation of number of cases needed for statistical significance cannot be made as there is neither data on terizidone, PAS serum levels nor on standard deviations in children of different age groups. For reasons of practicability and according to the incidence of a therapy regimen containing terizidone and PAS in childhood tuberculosis, we plan to enrole up to 30 children for terizidone, up to 10 children for PAS and up to 40 adults for PAS, however this relatively small sample size may have less statistical power.

Inclusion criteria

Children between 2 month and 13 years of age and adults older than 18 years who require treatment with second line anti-TB drugs terizidone and PAS and are admitted to one of the participating hospitals, who are medically stable and accompanied by a parent or legal guardian who has given written, informed consent for the participation of the child in the study. Patients included in another study **(Schaaf et al)** involving PK of ethionamide may also be included in the present protocol

Exclusion criteria

Children who are medically unstable such as those with severe anaemia, severe malnutrition, diarrhea and dehydration or other medical condition such as to make the drawing of blood inadvisable, or whose parents or legal guardian are unwilling to give permission for their children to participate in the study.

Procedure for *in-vivo* study

The serum concentration of terizidone will be determined at time **0, -1, -2, -3, -4-, -6, -** and **24** hours after dosing in groups of up to 10 children from each age group, in children 3 months -<2 years, 2-<6 years, and 6-12 years of age receiving the currently recomended dosage. This methodology requires **1 ml of blood** for each assay and **a total of 7 ml of blood** will thus be drawn during each assessment.

The serum concentrations of PAS will be determined at time **0, - 2, - 4, - 6, - 8, - and 12 or 24** hours in one group of up to 10 children aged between 3 months and 13 years after receiving a single dose per day. In adults, blood sampling times will be **0, -2, -3, -4, -5, -6, -8 and 12 or 24** hours. Blood sampling will be repeated after 2 to 4 weeks after receiving a multiple dose per day (twice-daily). The dose will be kept refrigerated and will be given with an orange juice under observation from the researcher. This methodology requires **1 ml of blood** for each assay and **a total of 12 ml of blood** will thus be drawn for both assessments in children and **a total of 20 ml** in adults.

These amounts does not exceed the allowable sample volume of 9.6, 36, 96 ml for the full term neonates, children (3 years) and children (12 years), respectively **(Abdel-Rahman et al., 2007).** However, considerations will be given to patients with diarrhea, severe anaemia, severe malnutrition or any other medical conditions that

restrict blood sampling.

In the paediatric population, an anesthetic patch will be applied for 45 minutes before inserting an indwelling catheter that will be used so that venipunctures are reduced throughout the investigation.
Serum concentrations will be determined by an established high performance liquid chromatography with mass spectrometry (HPLC-MS) methodology.

Procedure for *in-vitro* metabolism

The test compound will be incubated at concentrations with the enzyme source (Human liver microsomes (HLM), and S9 fractions) with appropriate positive and negative controls. At varying time intervals, the reactions will be terminated. The time spacing and replicate number will be according to the standardized procedures. The amount of parent drug remaining in the incubation media will be analyzed by HPLC-MS.
The current procedure will involve the incubation of substrates for specific isoenzymes with the test compounds and the enzyme source (Human liver microsomes) to determine the effects of these drugs to different metabolizing enzymes.

Procedure for pharmacogenetics assays

The blood cell pellet obtained from blood samples taken for pharmacokinetic assays will be stored frozen at -40^{o}C or below. In an initial stage it is planned to determine the genotype for both types of N-acetyl-transferase (NAT-1 and NAT-2). Samples may also be analysed for other proteins involved in drug elimination (such as P-glycoprotein) at a later stage.

Data management and Statistical analysis

Microsoft Excel will be used to capture the data and STATISTICA version 8 (StatSoft Inc. (2008) STATISTICA (data analysis software systems. will be used to analyse the data. Data will be collected on case report forms (CRF) and study flowcharts and then entered into an Access or SPSS database.
Summary statistics will be used to describe the variables. Distribution of variables will be presented as histograms and or as frequency tables. Medians or means will be used as the measures of central location for ordinal and continuous responses and standard deviations and quartiles as indicators of spread. The relationship between

continuous response variables and nominal input variables will be analysed using appropriate analysis of variance (ANOVA). When ordinal response variables are compared versus a nominal input variable, non-parametric ANOVA methods will be used. For completely randomized designs the Mann-Whitney test or the Kruskal-Wallis test will be used and for repeated measures the Wilcoxon- or Friedman tests will be used.

A population pharmacokinetic analysis will also be conducted to determine the pharmacokinetic effects of different covariates and to quantify the covariates contributing to the studied drugs' clearance and volume of distribution population parameters including variability in order to establish an initial estimate of the major covariates required for dosing predictions.

Ethical consideration

The study will be conducted according to the principles of Good Clinical Practice, South African and internationally accepted ethical standards taking into account the Declaration of Helsinki and International Conference on Harmonisation (ICH) guidelines.

All patients referred to the hospital for treatment might participate if they are eligible and a written informed consent (for children: by parents or legal guardians) is given. Before enrolment, children older than 7 years will be asked to assent after being fully informed about the rationale, procedure, data management as well as potential benefits and risks of the study.

The patients participating in the study will not directly benefit from the study; however, the study will provide important information for the optimal treatment and the use of terizidone and PAS in childhood tuberculosis.

This study is in the interest of improving medical care for children with tuberculosis in need of the second line drugs, terizidone and PAS. No alteration in the prescribed treatment of the children will be made other than that prescribed by the attending physicians.

The consent form will be available in English, Afrikaans and isiXhosa, the parents will be informed of the possibility to revoke informed consent even though it was made in writing. There will be no financial incentive offered for study participation.

Blood samples for measurements of serum levels and additional parameters will be collected through a permanent angiocatheter that will be inserted at the beginning of each study day to minimize the number of necessary venipunctures.

Obtained data will be collected in a database using a unique anonymous subject code for each patient. Identified information linked to the study code will be recorded separately. The confidentiality of all information of a personal nature will be maintained and ensured at all times. Only the researcher and authorized officials affiliated to control bodies will be allowed access to personal information.

It is the intention of the researcher to present and publish the findings of the study in an appropriate medical journal without, as indicated, divulging any information of a personal nature.

Risks associated with participation in the study

The participants will be treated as usual by their attending physician. The investigators will not in any way alter their drug therapy. The risks are therefore those associated with venipuncture. These include pain, bruising and bleeding at the site of IV cannula insertion.

To minimize these risks, an indwelling IV cannula will be inserted so that repeat venipuncture is minimized and a topical anaesthetic gel will be used. These symptoms usually resolve spontaneously or may require the application of gentle pressure to the area for a few minutes after cannula removal.

A further risk is the drawing of blood in children who are anaemic or dehydrated. To prevent this**,** children who are medically unstable such as those with severe anaemia, severe malnutrition, diarrhea and dehydration or other medical condition such as to make the drawing of blood inadvisable will be excluded from the study.

Furthermore, the amount of blood drawn will be limited to 8ml in total, which is less than the upper limit recommended in guidelines (**Abdel-Rahman et al., 2007**).

Feasibility of the study

The participating hospitals admit good number of children per year (approximately 120-130) of whom great majority have either drug-resistant tuberculosis and require second line anti tuberculosis agents. It should therefore be possible to enroll the proposed number of patients at these facilities within a time a year. Furthermore, the

Division of Pharmacology has a long standing and extensive experience in conducting pharmacokinetic studies in childhood tuberculosis. It has previously been involved in studies which characterized the pharmacokinetic disposition of isoniazid in children and has produced refined the methodology for determining the serum concentrations of several antituberculosis agents.

For the in vitro study we are collaborating with experts from Synexa group, South Africa, who have considerable experience in such kind of laboratory work; the reagents for the in vitro study are readily available commercially.

Conclusion

The data generated in this study will be used as guidance for future studies involving much larger populations. We plan to present the data at congresses and to write this up for publications.

Time plan

We have planned to submit the protocol between August-September 2009 and have full Ethics approval for the study to start shortly thereafter. The aim is to collect data according to the protocol within a year. Data entry analysis and writing of the manuscript could be completed in 2 months. It would be possible to aim at the final manuscript at the end of year 2010 for the final master's thesis presentation. At the time of the submission of this amendment, the clinical project is ongoing (paediatric population).

The in vitro study will have a specific timeline, its implementation will commence after the approval from the ethical committee and the arrival of the required study materials.

Funding and budget:

Partial funding from the departmental funds is readily available. In addition, funding requests/ applications have been submitted to Medical Research Council and Harry Crossley Foundation and we await for feedback and possible approval. The budget summary is as indicated in the chart below:

Table 1: Budget

	Unit cost (R)	No. of units	Sub-total (R)
Consumables			
Blood collection and specimens	75	180	13 500
Micro centrifuge polypyrolene tubes	950	1	950
Reagents			
Liver microsomes	1660	25	41 500
S9 fractions	650	20	13 000
Co-factors (*NADPH, UDPGA*)			9 000
Other reagents (*DMSO, Phosphate buffer, Methanol, Acetonitrile)*			4 000
Substrates			20 000
Laboratory analyses			
Development and Validation of assays			10 000
Analysis of specimens	800	180	144 000
Travel			
Parents/ caregivers to and from the hospital	100	30	3 000
Cost for courier services	-	-	8 000
Total			**266 950**

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Appendix

Terms and definitions relating to tuberculosis and drug resistance

- ***Drug resistant tuberculosis***-Tuberculosis that is resistant to any first line antituberculosis drug
- ***Multidrug resistant tuberculosis (MDR-TB)***-Tuberculosis that is resistant to at least isoniazid and rifampicin
- ***Extensively drug resistant tuberculosis (XDR-TB)***-Tuberculosis that is resistant to at least isoniazid and rifampicin and also to a fluoroquinolone and a second line injectable agent (amikacin, capreomycin, or kanamycin)
- ***Drug resistance in new tuberculosis cases (primary drug resistance)***-Drug resistant tuberculosis in a person with no history of tuberculosis treatment, implying they were infected with a resistant organism. This reflects person to person transmission of drug resistant tuberculosis
- ***Drug resistance among previously treated cases ("acquired" drug resistance*)-**Drug resistant tuberculosis in a person with a history of tuberculosis treatment. This reflects drug resistance acquired during tuberculosis treatment but may also reflect infection or reinfection with a resistant organism (**Grant et al., 2008)**

The standardized regimen for treatment of multidrug-resistant TB in South Africa is as shown in the table below:

Standardized Regimen for Treatment of Multidrug-Resistant TB(MDR-TB) in SA **Treatment should be started in a referral centre and be adjusted according to a patient weight**						
Pre-treatment body weight	**Intensive phase: 4 months** **Doses given daily**					
	Kanamycin	**Ethionamide**	**PZA**	**Ofloxacin**	**Ethambutol or terizidone**	
< 50 kg 50-65 ≥ 65	750 mg 1000 mg 1000 mg	500 mg 750 mg 750 mg	1000 mg 1500 mg 2000 mg	600 mg 600 mg 800 mg	800mg 1200mg 1200 mg	750 mg 750 mg 750 mg
Pre-treatment body weight	**Continuation phase 12-18 months depending on culture conversion** **Doses give daily**					
	Ethionamide		**Ofloxacin**		**Ethambutol or terizidone**	
< 50 kg 50-65 kg ≥ 65 kg	500 mg 750 mg 750 mg		60 mg 600 mg 800 mg		800 mg 1200 mg 1200 mg	500 mg 750 mg 750 mg

Ethambutol is used if strain still susceptible. Terizidone is used if strain resistant to ethambutol. Reduce terizidone dose to 500 mg if weight ≤ 35 kg. Pyridoxine (Vit B6) 150 mg to be given to patients on terizidone. Some provinces may be using cycloserine instead of terizidone. **(Begley, 2007)**

Appendix C: Protocol of Study 2

PHARMACOKINETICS OF TWICE DAILY VS. ONCE DAILY DOSING WITH GRANULAR SLOW-RELEASE PARA-AMINOSALICYLIC ACID IN ADULTS ON SECOND-LINE ANTI-TUBERCULOSIS AND ANTIRETROVIRAL TREATMENT

PLACE OF RESEARCH:

Division of Pharmacology, Faculty of Health Science, University of Stellenbosch, Tygerberg

INVESTIGATOR

Lizanne de Kock1

CO-INVESTIGATORS:

Prof AH Diacon3

Prof PR Donald MD2

Dr K Prescott MFPM1

Dr HI Seifart PhD 1

Prof B Rosenkranz MD PhD FFPM 1

Dr C Werely

1Division of Pharmacology, Department of Medicine, Faculty of Health Science

2 Department of Paediatrics and Child Health and Desmond Tutu TB Centre

3Department of Molecular Biology and Human Genetics, and the MRC Centre for Molecular and Cellular Biology, DST/NRF Centre of Excellence for Biomedical TB research

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APPENDICES

1. PROTOCOL SYNOPSIS

Title

Pharmacokinetics of twice daily vs. once daily dosing with granular slow-release para-aminosalicylic acid in adults on second-line anti-tuberculosis and antiretroviral treatment

Introduction

Extensive drug resistant tuberculosis (XDR-TB) is difficult to treat and a high mortality rate has been reported. These patients may require second-line treatment which includes para-aminosalicylic acid (PAS). Despite being the oldest effective TB drug, little is known about the pharmacokinetics of PAS and interactions with other drugs, particularly in the face of antiretroviral drug regimens and using the new granular slow-release PAS (GSR-PAS). Although, interactions have not been reported, the pill burden prescribed to multi-drug resistant tuberculosis (MDR-TB) and XDR tuberculosis patients with HIV may increase the potential for interactions. Several efforts are underway not only to improve the management of MDR-TB cases co-infected with HIV, but also to reduce the daily drug regimen, pill burden and duration of therapy. In the face of large pill burden due to multiple drugs being used to treat both TB and AIDS, and a treatment programme that relies on directly observed treatment (DOT), a once-daily dosing regimen for PAS is preferable rather than the twice-daily regimen being used at present. As PAS is increasingly being used in the second-line treatment of TB, this study aims to add to our understanding of the pharmacokinetic profile of PAS in adults on multiple drug regimens, including patients who are also on antiretroviral drug regimens and those who are not.

Primary objective is to investigate the pharmacokinetics of PAS given once daily vs. twice daily and to establish whether once daily dosing will result in concentrations above the MIC of M tuberculosis for a period comparable to twice daily dosing in adult MDR/XDR-TB patients.

Secondary objectives: To investigate the safety and tolerability of PAS when given once daily vs. twice daily; to investigate the pharmacokinetics of PAS in patients on antiretroviral treatment and those not on antiretroviral treatment; and to investigate the pharmacokinetic effects of genetic variations of the genes involved in PAS

metabolism (NAT 1 and NAT 2)

Study design and methodology

This is a randomized, two-period, open-label cross-over study which will investigate and compare the pharmacokinetics and tolerability of the two different methods of PAS dosing, once-daily vs. twice-daily, in adult patients with MDR- or XDR-TB. The proposed study can be completed only in a population with high prevalence of drug resistant disease and HIV. Therefore, the study will utilize a hospital-based strategy to recruit adults routinely screened for drug resistant tuberculosis and treated for the disease. Forty eight adults (aged ≥ 18) at Brooklyn Hospital for Chest Diseases and/or D.P. Marais Hospital, Cape Town, South Africa will be enrolled in the study. These patients will be equally divided into two groups: Those on antiretroviral treatment (ART) and those not on ART (24 patients in each group). Patients in each group will be randomized to receive either 2x 4g GSR-PAS (n = 2x12) or 1x8g GSR-PAS (n =2x12) for 8 days. Samples will be collected for pharmacokinetic analysis over a 24 hour period on Day 8. Thereafter, the regimens will be crossed over and these regimens will be followed for another 8 days (Days 9 to 15). Blood samples will again be taken over a 24 hour period on Day 16. The duration of the study for an individual subject will be 31 days. Dosage regimens, dosing time and blood sampling times will be recorded for each patient. Three millilitre blood specimens will be collected through a catheter inserted into a forearm vein, pre-dose and at t0, 1, 2, 3, 4, 6, 8, 10, 12 (pre-dose) and 24 hours after dosing. Therefore, 30 ml blood will collected for each pharmacokinetic sampling day. An additional blood sample (3 ml) will be collected in an EDTA tube for genetic analysis. If not already known, blood for HIV testing will also be obtained.

Sample Analysis

Samples will be analysed for PAS by a high-performance liquid chromatography (HPLC) MS/MS assay using binary HPLC (Agilent Series 1100 HPLC, Agilent Technologies, Waldbronn, Germany) equipped with an Agilent Zorbax analytical column (150mm x 2.1 mm, 3.5 µm particle size.

Safety monitoring

Safety and tolerability will be assessed by health assessments including physical

examinations, Visual analogue scales (VAS), clinical laboratory results, reporting of adverse events and vital signs. Subjects will be interviewed at the beginning and at the end of the study and monitored throughout the study. If clinically indicated, additional tests will be conducted as required.

Risks and Benefits

The participants will be treated as usual by the attending physician. The investigators will not alter their drug therapy, except for the PAS dosing regimen during the study period. The patients may experience pain, bruising and/or bleeding at the side of 20-gauge angiocatheter insertion. In addition, there is a risk that unexpected side-affects may occur and that the once-daily dosage may not be as effective as the standard dosage. However, it has never been demonstrated in any trial that twice or thrice daily dosing gives better results than once daily dosing. It is thought that the plasma concentration must be continuously above known minimum inhibitory concentrations (MIC= 1µg/ml) to be effective. Therefore, the side-affects will be monitored and the patient will be made aware of the risks of the study.

Ethical Considerations

The project will be conducted accordingly to South African laws and regulations including South African GCP and to the Declaration of Helsinki. Blood samples will be collected through an indwelling catheter which will be inserted at the beginning of each pharmacokinetic study day to minimise the number of necessary punctures. A qualified research nurse will insert the catheter and the patients may experience minor pain or bruising at the site. The data and information of each patient will be concealed by the use of a unique subject code and identified information linked to study code will be recorded separately. The confidentiality will be maintained at all times. Only the researcher and authorized officials affiliated to control bodies will be allowed access to personal information.

2. LITERATURE REVIEW

Multi-drug resistant tuberculosis (MDR-TB) caused by strains of M. tuberculosis resistant to first-line tuberculosis (TB) drugs, rifampicin and isoniazid, has emerged as an increasing threat world-wide 1, 2. Extensively drug-resistant (XDR) isolates are in addition, resistant to second-line drugs, i.e. fluoroquinolones and injectable second-line drugs such as kanamycin (KM), amikacin (AM) and capreomycin (CM) 1, 3. The emergence of these drug-resistant (DR) strains largely results from deficiencies in TB case- and program-management 1.

XDR-TB is difficult to treat and a high mortality rate has been reported. According to the South African MDR-TB Guidelines (2011), depending on the resistance pattern of the isolates, these patients may require treatment with the following second-line drugs: capreomycin, moxifloxacin, ethionamide, terizidone, pyrazinamide, clofazimine and para-aminosalicylic acid (PAS). These second-line drugs are less effective, more toxic and more costly than drug-susceptible TB treatment 2. Little is known about the pharmacodynamics and pharmacokinetics of these drugs, but especially the new slow-release preparation of PAS.

PAS

PAS was used to treat tuberculosis for the first time in 1944 4 and formed part of the standard treatment for TB for several decades until more effective first-line drugs for example, rifampicin were introduced. In 1992, PAS was re-introduced in the USA for management of MDR-TB and later for XDR-TB 5 and is increasingly used in South Africa for treatment of DR-TB.

PAS is a bacteriostatic agent valuable in preventing resistance to other drugs 6. It has been described as less tolerable than the other second-line drugs and has a short half-life. After re-introduction as a treatment for MDR-TB, a granular slow-release formulation (GSR-PAS) was developed, which demonstrated an improved gastrointestinal tolerance and a longer half-life than the immediate-release sodium PAS tablet 7. The maximum serum concentration is reached after 6 hours following GSR-PAS administration 8

Despite the above reservations about once daily dosing it is of interest that several early studies comparing once daily and intermittent daily GSR-PAS dosing found that

adult patients tolerated once daily dosing better than multiple doses. 9, 10, 11 It was also reported that a single daily GSR-PAS dose was clinically as effective as divided daily doses of GSR-PAS, both in TB patients 9 and experimental animals.12 When the early bactericidal activity of GSR-PAS in a single daily dose of 15 g over the first two days of treatment was evaluated in a small group of pulmonary TB patients it was second only to that of isoniazid suggesting significant early bactericidal activity at higher concentrations.13

Peloquin et al. conducted a study with GSR-PAS (Paser; Jacobus Pharmaceuticals, Princeton, NJ) in a small number of patients and reported that serum concentrations in excess of the MIC were achieved throughout the dosing interval of the granules 7. After the 22nd dose, a median serum concentration of nearly 3 times higher than the initial concentration was reported, which indicated an accumulation of GSR-PAS.

In another study by Peloquin et al., once daily dosing in comparison to a twice daily dosing regimen of GSR-PAS was investigated, and concentrations above improved minimum inhibitory concentrations (MIC= 1µg/ml) were reported to be longer for the 4g twice daily than the 4g once daily regimen8. The granules were generally well tolerated in this study; however a few patients reported nausea and gastrointestinal discomfort, which can be ascribed to the high serum concentrations (80-90µg/ml). Higher pH in the patient's stomach can increase the rate of release of PAS from granules which can cause an increased serum concentration, but also a more rapid elimination of PAS. The higher pH is possibly due to the basic food or beverages patients consumed near their dosing time. Although twice daily dosing is associated with a longer period above MIC, multiple daily doses are not practical in all settings especially not in resource-limited settings with notably poor patient compliance and a need for directly observed treatment (DOT). Peloquin's studies were also carried out on MDR-TB treated patients and did not include those treated with anti-retroviral treatment (ART) and other anti-tuberculosis agents.

Despite being the oldest effective TB drug, little is known about the pharmacokinetics of PAS and interactions with other drugs, but especially other anti-tuberculosis and antiretroviral treatments. Although, interactions have not been reported, the pill burden prescribed to MDR and XDR tuberculosis patients with HIV may increase the potential for interactions.

In addition to drug interactions, genetic variations may influence an individual's ability

to utilize and tolerate different treatments. Gene variations involved in absorption, distribution and metabolism of drugs are often studied in this regard. Variants in N-acetyltransferase 2 (NAT2) have long been recognized to modulate toxicity produced by the anti-tuberculosis drug isoniazid (INH) 14. PAS is metabolised by acetylation, where more than 50% of the drug is acetylated 15. N-acetyltransferase 1 (NAT 1) is responsible for the acetylation of PAS. NAT 1 is also known to be polymorphic but is usually thought to have a relatively minor effect on PAS pharmacokinetics 16.

This is a randomized, two-period, open-label cross-over study which will investigate and compare the pharmacokinetics and tolerability of the two different methods of PAS dosing, once-daily vs. twice-daily, in adult patients with MDR- or XDR-TB, including patients who are also on ART and those who are not, in order to investigate the possible interactions with commonly used ARV regimens.

3. RATIONALE FOR STUDY DESIGN

Little is known about the pharmacokinetics of PAS in treatment of adults requiring second-line tuberculosis treatment, particularly in the face of antiretroviral drug regimens and using the new slow-release preparation. Several efforts are underway not only to improve the management of MDR-TB cases co-infected with HIV, but also to reduce the daily drug regimen, pill burden and duration of therapy. In the face of large pill burden due to multiple drugs being used to treat both TB and AIDS, and a treatment programme that relies on DOT, a once-daily dosing regimen for PAS is preferable rather than the twice-daily regimen being used at present. As PAS is increasingly being used in the second-line treatment of TB, this study aims to add to our understanding of the pharmacokinetic profile of PAS in adults on multiple drug regimens, including patients who are also on antiretroviral drug regimens and those who are not.

Genetic variations

Significant individual variations in the tolerability and effects of PAS have been reported. This may be due to genetic factors. N-acetyltransferase (NAT) is responsible for the metabolism of PAS and will therefore be investigated in the study. Formal evaluation by genotyping of the effects of NAT1 and NAT2 enzymes on the pharmacokinetics of PAS has never been undertaken.

4. OBJECTIVES

4.1. Primary objective is to investigate the pharmacokinetics of PAS given once daily vs. twice daily and to establish whether once daily dosing will result in concentrations above the MIC of M tuberculosis for a period comparable to twice daily dosing in adult MDR/XDR-TB patients.

4.2. Secondary objectives:

– To investigate the safety and tolerability of PAS when given once daily vs. twice daily

– To investigate the pharmacokinetics of PAS in patients on antiretroviral treatment and those not on antiretroviral treatment

– To investigate the pharmacokinetic effects of genetic variations of the genes involved in PAS metabolism (NAT 1 and NAT 2)

5. STUDY DESIGN AND METHODS

This is a randomized, two-period, open-label cross-over study which will investigate and compare the pharmacokinetics and tolerability of the two different methods of PAS dosing, once-daily vs. twice-daily, in adult patients with MDR- or XDR-TB. The proposed study can be completed only in a population with high prevalence of DR disease and HIV. Therefore, the study will utilize a hospital-based strategy to recruit adults routinely screened for DR-TB and treated for the disease.

Forty eight adults (aged≥ 18) at Brooklyn Hospital for Chest Diseases and/or D.P. Marais Hospital, Cape Town, South Africa will be enrolled in the study. These patients will be equally divided into two groups: Those on ART and those not on ART (24 patients in each group). Patients in each group will be randomized to receive either 2x 4g GSR-PAS (n = 2x12) or 1x 8g GSR-PAS (n =2x12) for 8 days. Balanced allocation will mean that 12 patients on ART and 12 patients not on ART will receive each regimen in both Treatment Periods 1 and 2.

Samples will be collected for pharmacokinetic analysis over a 24 hour period on Day 8. Thereafter, the regimens will be crossed over, with those who received 2x4g GSR-PAS, then receiving 1x 8g GSR-PAS and those who received 1x 8g GSR-PAS then

receiving 2x 4g GSR-PAS. These regimens will be followed for another 8 days (Days 9 to 16). Blood samples will again be taken over a 24 hour period on Day 16. The duration of the study for an individual subject will be 31 days. Day -7 to -1: Screening and baseline measurements; Day 0: Subjects will be officially enrolled on Day 0 and will be randomised to one of the treatment regimens; Day 1-8: Continue the dosing for 8 days; Day 8: Pharmacokinetic samples will be taken; Day 9: Cross-over; Days 8-16: continue the dosing; Day 16: Pharmacokinetic samples will be taken; Day 23: follow- up. Figure 1 is a schematic illustration of the study and table 1 shows the study events.

Figure 1 Schematic illustration

<table>
<tr><td>Days -7 to -1</td><td>Day 0</td><td>Days 1-7</td><td colspan="2">Day 8</td><td>Day 9- 15</td><td colspan="2">Day 16</td><td>Day 17-23</td></tr>
<tr><td rowspan="4">Screening</td><td rowspan="4">Randomisation</td><td colspan="3">Continuous dosing with PAS during this period</td><td colspan="3">Cross-over; Continuous dosing with PAS during this period</td><td rowspan="4">Follow-up</td></tr>
<tr><td>No PK samples will be taken for 7 days</td><td colspan="2">PK sampling</td><td>No PK samples will be taken for 7 days</td><td colspan="2">PK sampling</td></tr>
<tr><td>2x 4g GSR-PAS</td><td>12 patients</td><td>12 patients</td><td>1x 8g GSR-PAS</td><td>12 patients</td><td>12 patients</td></tr>
<tr><td>1x 8g GSR-PAS</td><td>12 patients</td><td>12 patients</td><td>2x 4g GSR-PAS</td><td>12 patients</td><td>12 patients</td></tr>
<tr><td colspan="9">2x 4g GSR-PAS: 8 days of dosing with PAS 4 g twice daily; 1x 8g GSR-PAS: 8 days of dosing with PAS 8g once daily; ■ Patients on ARVs; □ Patients not on ARVs</td></tr>
</table>

Table 1 Events table

Test	-7 to -1	1 to 7	8	9	10 to 15	16	17 to 22	23
Screening	**X**							
Randomisation		**X**						
PAS routine	**X**						**X**	**X**
GSR-PAS 2x4g*		**X**	**X**	**(X)**	**(X)**	**(X)**		
GSR-PAS 1x8g*		**(X)**	**(X)**	**X**	**X**	**X**		
PK sampling			**X**			**X**		
Cross over*				**X**				
Follow up								**X**

*** Patients will be randomized to start with either treatment and be switched to the alternative treatment at cross over day.**

5.1. Sample size

The sample size chosen for this study was based on precedent set by other pharmacokinetic studies of similar nature and not on statistical considerations. However, the sample size is more than twice the sample size used in other pharmacokinetic studies in order to account for known variability in the pharmacokinetics of PAS, and the potential for patients to drop out.

5.2. Subject criteria

Eligible: Adults routinely screened for MDR-TB and XDR and treated for disease (N= 48) at Brooklyn Hospital for Chest Diseases and/or D.P. Marais Hospital, Cape Town, South Africa, the following will be eligible for enrolment.

5.2.1. Inclusion criteria

The following criteria must be met to participate in this study:

- Males and females, aged ≥18 years

- Written informed consent; if patients agree to take part in the genetic sub-study, a separate informed consent must be obtained for this sub-study
- Known HIV status or willingness to undergo HIV testing in the presence of unknown status. A negative test result will be accepted up to 4 weeks
- Participants must have been clinically stable on an unchanged regimen including PAS as part of their routine treatment for 14 days or more
- The subjects must assent for all aspects of the study and be able to give written informed consent for participation in the study before enrolment

5.2.2. Exclusion criteria

Subjects will not be included in the study if they meet any of the following criteria:

- Informed consent for participation or consent for HIV testing not obtained if no HIV test is available
- Any concomitant illness that according to the investigator makes participation inadvisable because of potential interference with study variables or increase risk to the patient
- Planned change in TB or HIV related treatment during the study period

5.3. Study procedures

5.3.1. Informed Consent

Before enrolment into the study and any study procedures will be performed, the participant has to give written informed consent for the study. (See appendices for consent forms)

5.3.2. Baseline Procedures

The following measures and data will be obtained from the patients at baseline (enrolment): HIV status and HIV-associated immunity, TB disease status and disease spectrum and chest radiograph (CXR), mycobacterial culture and drug susceptibility test DST pattern, concurrent illness, concomitant medication, age, Body Mass Index (BMI), blood pressure, liver function, renal function, thyroid function, haematology, race and gender.

Note: Race is an important aspect, as drug response is different among different

ethnic groups. Local race categories will include African, Indian, Caucasian and South African Coloured (mixed ethnicity). Gender will be categorized as male or female.

5.3.3. Physical Examination

Physical examination will take place at screening and on Days 7 and 15 during the study, as well as a follow-up physical examination.

5.3.4. Treatment Procedures

The patients will follow their current drug regimens for their tuberculosis treatment; and the drugs and dosage will be carefully recorded. All HIV-infected patients will be on ARVs. An hour before eating, PAS will be given to all patients with 250 ml of orange juice and observed to ensure that they drink all of the juice at the time of dosing

The patients will already be on PAS (≥2 weeks TB treatment), therefore only the daily regimen for this drug will change, which they must follow for seven days before the first pharmacokinetic sampling will take place. The regimens (once or twice daily) will be allocated to the subjects according to an agreed randomisation schedule. Adherence to PAS and other medicines will be well controlled and recorded.

5.3.5. Dosage and Administration

2x4g GSR-PAS: Twice Daily dosing- Patients will receive a 12-hourly dose (4g) oral PAS (PASER® granules) regimen with other already prescribed medicines (See appendices for package insert:

1x8g GSR-PAS: Once Daily dosing- Patients will receive a single (8g) oral PAS (PASER® granules) dose once daily along with other already prescribed medicines (ARVs and other anti-TB drugs) according to the usual dosing schedule.

An hour prior to food, PAS will be given with yoghurt or a glass of orange juice under direct observation. The acidic beverage prevents early release of the drug in the stomach 8.

5.3.6. Pharmacokinetic (PK) sampling

The following pharmacokinetic parameters of PAS will be determined by using non-compartmental analysis: Maximum observed concentration (Cmax); time at which Cmax occurred (Tmax); area under the plasma concentration-time curve (AUC); Minimum observed concentration Cmin.

The blood samples for the PK assessments will be taken at the following time points: pre-dose (t0), 1, 2, 3, 4, 6, 8, 10, 12 (pre-dose) and 24 hours.

5.3.7. Sample collection

Dosage regimens, dosing time and blood sampling times will be recorded for each patient. Three millilitre blood specimens will be collected through a catheter inserted into a forearm vein, pre-dose and at t0, 1, 2, 3, 4, 6, 8, 10, 12 (pre-dose) and 24 hours after dosing. Therefore, 30 ml blood will collected for each pharmacokinetic sampling day. The blood will also be used for genetic analysis and informed consent will be obtained separately for genotyping. If not already known, blood for HIV testing will also be obtained.

5.3.8. Safety monitoring

Safety and tolerability will be assessed by health assessment including physical examinations, self-rating Visual Analogue Scales (VAS), clinical laboratory results, reporting of adverse events and vital signs. Subjects will be interviewed at the beginning and at the end of the study and monitored throughout the study. If clinically indicated, additional tests will be conducted as required.

Concurrent illness

Concurrent illnesses which may have an impact on evaluation of toxicity will be documented before and during the study, such as viral hepatitis, central nervous system disease, HIV-related target organ disease (particularly renal and hepatic impairment) and others.

Concomitant medication

All concomitant medications will be documented, including-but not limited co-trimoxazole (TMP/SMX); multivitamin supplementation, analgesics, antifungals, anthelmintics, antiretroviral treatment, anti-tuberculosis treatment and other antibiotics. Should anti-nausea or anti-diarrhoeal medications are required during the study; these will also be recorded and included in the adverse event reporting where appropriate.

Self-rating scales

The most common adverse drug reactions reported for PAS are gastrointestinal intolerance manifested by vomiting, diarrhoea, nausea, and abdominal pain. These adverse events will be rated by the participant with self-rating Visual Analogue Scales (VAS). VAS scales are well validated for assessment of symptoms in gastrointestinal conditions 17. The VAS scales will be used to rate the gastrointestinal discomfort using scales for: bloating, vomiting, nausea, diarrhoea and abdominal pain. The scales will be 10-point scales, with the far left indicating no symptoms and far right as severe symptoms. The participants will be asked to complete the self-rating VAS scales on a daily basis during the study period. (See appendices for VAS scales.

Clinical laboratory results

Biochemical parameters for liver, renal, and thyroid function, and haematological parameters will be tested at baseline. Thyroid function will be measured at the end of the study.

Table 2 Clinical Laboratory Tests

Haematology	Plasma Chemistry	Urinalysis
MCV	ALT	pH
MCHC	Alkaline phosphatase	Specific gravity
Red blood cells	AST	Ketones
White blood cells	Bilirubin, total and direct	Protein
Differential white cell count	Creatinine	Glucose
Hemoglobin	Urea	Blood
Haematocrit	Creatine kinase	
Platelets	Potassium	
	Sodium	
	Glucose	
	TSH	

6. SAMPLE ANALYSIS

Samples will be analysed for PAS by a high-performance liquid chromatography (HPLC) MS/MS assay using binary HPLC (Agilent Series 1100 HPLC, Agilent Technologies, Waldbronn, Germany) equipped with an Agilent Zorbax analytical column (150mm x 2.1 mm, 3.5 µm particle size

7. GENETIC ANALYSIS

DNA will be extracted from the blood sample obtained for genetic analysis. Variations in the N-acetyltransferase (NAT1 and NAT2) will be determined through polymerase chain reaction (PCR) and sequencing procedure.

8. PHARMACOKINETIC ANALYSIS

Descriptive statistics (number of subjects, mean, CV%, SD, percent co-efficient of variation, 25th percentile median, 75th percentile, minimum, and maximum) will be used to summarize the plasma concentrations of PAS at each scheduled time-point. Linear and semi-logarithmic plots of the mean and individual plasma concentration-time curves of the analytes on Day 1 and Day 5 will be provided. Individual subject plasma concentration data will be listed.

Actual post-dose sampling times, rather than scheduled sampling times will be used in the derivation of plasma PK parameters. For ease of presentation, scheduled sampling times will be used to present results in tables, listings and figures.

The following PK parameters will be determined for each subject:

AUC (0-24): Area under the concentration-time curve from time 0 to 24 hours post-dose on Days 8 and 16

Cmax: Maximum observed plasma concentration from time 0 to 12 hours post dose on Days 8 and 16

Cmin: Minimum observed plasma concentration just prior to dosing on Days 8 and 16

Tmax: Time to reach Cmax on Days 8 and 16

9. STATISTICAL ANALYSIS

The sample size chosen for this study was based on precedent set by other pharmacokinetic studies of similar nature and not on statistical considerations, as very little data is known.

Subjects will receive a subject number at the time of signing the informed consent form and will be randomized according to the numbers to receive 2x 4g GSR-PAS or 1x 8g GSR-PAS. The subject numbers will be used in all study documentations. Subject numbers will not be re-used if a subject withdraws. The statistician will draw up a randomisation schedule using blocks of 4 to balance the baseline to ensure that equal numbers of patients on ART and those who are not on ART are allocated to each Regimen.

Each variable will be summarised using standard summary statistics, according to the regimen sequence. AUC, Cmin and Cmax will be analysed, after log transformation, using an analysis of variance model (ANOVA), fitting for the effects of subject, period and regimen (once-daily vs. twice daily) as a random effect. The results of analysis will be presented in terms of the geometric least squares means for each regimen, the ratio of the geometric least squares means and the corresponding 95% confidence interval.

The median difference between each treatment in Tmax will be assessed non-parametrically on untransformed data; the difference between the 2 being analysed by use of a Wilcoxon signed rank test. All safety and tolerability data will be

presented with descriptive statistics.

10. TIME PLAN

Jan 2012	Feb 2012	March 2012	Apr 2012	May 2012	Jun 2012	Jul 2012	Aug 2012	Sept 2012	Oct 2012	Nov 2012
Ethic approval			Start Study		Data analysis and writing up				Prepare for submission	Submit thesis

11. ETHICAL CONSIDERATIONS

The project will be conducted accordingly to South African laws and regulations including South African GCP and to the Declaration of Helsinki.

The consent forms will be available in English, Afrikaans and isiXhosa. Participants will be compensation for inconvenience (R150 per visit).

Blood samples will be collected through an indwelling catheter which will be inserted at the beginning of each pharmacokinetic study day to minimise the number of necessary punctures. A qualified research nurse will insert the catheter and the patients may experience minor pain or bruising at the site.

The data and information of each patient will be concealed by the use of a unique subject code and identified information linked to study code will be recorded separately. The confidentiality will be maintained at all times. Only the researcher and authorized officials affiliated to control bodies will be allowed access to personal information.

12. RISKS AND BENEFITS

The participants will be treated as usual by the attending physician. The investigators will not alter their drug therapy, except for the PAS dosing regimen during the study period. The patients may experience pain, bruising and/or bleeding at the side of 20-gauge angiocatheter insertion. In addition, there is a risk that unexpected side-affects may occur and that the once-daily dosage may not be as effective as the standard dosage. However, it has never been demonstrated in any trial that twice or thrice daily dosing gives better results than once daily dosing. It is thought that the plasma

concentration must be continuously above known minimum inhibitory concentrations (MIC= 1µg/ml) to be effective. Therefore, the side-affects will be monitored and the patient will be made aware of the risks of the study.

Patients in the study will not directly benefit from the study; however the study will provide important information for the optimal dose regimen for future patients.

13. STRENGTHS AND LIMITATIONS

This is a cross-over study; therefore each participant will be his/her own control. The limitation of the study will be the relatively small number of participants in the study, and also that no sampling will take place during the night. PK samples will be collected pre-dose and up to 12 hours, with a final sample collected at 24 hours, which will be set as the endpoint.

14. REPORTING AND RESULTS

The results of the study will be presented at congresses/forums and included in scientific articles and a student thesis, without revealing the identity of the study participants.

15. RESOURCES AVAILABLE

The Brooklyn Hospital for Chest Diseases and/or D.P. Marais Hospital, Cape Town, South Africa hospital will be available for the study. A clinical research team will follow the pharmacokinetic study protocol accordingly.

16. FUNDING

Departmental funds will be available. In addition, funding will be available from NRF grants provided to Prof PR Donald.

17. BUDGET

BUDGET AND FUNDING			
	UNIT COST (R)	**NO OF UNITS**	**SUB-TOTAL**
Analysis			
Bio-analysis			R 288 000.00
CONSULTING SERVICES			
Statistical services			R 3 000.00
CLINICAL WORK			
	R 1 428.57 per participant	R 1,428.57X 8	R 68571.43
PERSONNEL			
Research nurse	150/hr.	R150X2X150	R 45 000.00
COMPENSATION			
Participant	R150 per visit	R 150 X 2 X 48	R 14 400.00
OTHER DIRECT COST			
Printing and copying			R 1 000.00
TOTAL EXPENDITURES			**R 419971.43**

18. REFERENCE

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Appendix D: Informed consent of Study 1

STELLENBOSCH UNIVERSITY

Participant information leaflet and consent form for use by parents/legal guardians

Title; Pharmacokinetics and dosing rationale of terizidone and para-aminosalicylic acid (PAS) in children and the evaluation of the in vitro metabolism of ethionamide, terizidone and PAS.

Reference number:

Principal Investigator: Prof Bernd Rosenkranz

Address: Department of Medicine, Division of Pharmacology, 7th Floor Clinical Building, Faculty of Health Sciences, Stellenbosch University, Francie van Zijl Drive, Tygerberg, 7505

Contact Numbers: Office phone; +27 21 938 9331

Mobile phone; +27-82-9550017

Your child is being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how your child could be involved. Also, your child's participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you or your child negatively in any way whatsoever. You are also free to withdraw him/her from the study at any point, even if you do initially agree to let him/her take part.

This study has been approved by the **Committee for Human Research at Stellenbosch University** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

Introduction

Tuberculosis (TB) is a very important health problem in South Africa and is often complicated by resistance to the commonly used medicines. When resistance is present other medicines (second-line medicines) must be used. These are not as effective as the medicines usually given for tuberculosis and they often have complications. The medicines used to treat drug resistant tuberculosis include para-ethionamide (ETH), terizidone /cycloserine (CS) and para-aminosalicylic acid (PAS). All of the second-line drugs are not very effective and it is important that they be given in the best possible dosage. There is no information regarding the amount of these medicines that get into the blood of children. Such information would make it possible for doctors to give much more accurate dosages of these medicines than is possible at present. This study aims to determine the amount of terizidone and PAS that gets into the blood of children of different ages following different doses of terizidone and PAS.

Before you decide if you want your child to be a part of this study, we want you to know about the study. You may choose not to be part of this study. No health care will be withheld for your child should you choose not to be part of the study. Your child will still receive the standard treatment for drug-resistant tuberculosis used at this hospital.

This is a consent form. It gives you information about this study. The study staff will talk with you about this information. You are free to ask questions about this study at any time. If you agree to allow your child to take part in this study, you will be asked to sign this consent form. You will get a copy to keep.

Why is this study being done?

The purpose of this study is to evaluate the absorption of the antituberculosis medicines, terizidone and PAS when given in different doses in children of different ages with drug-resistant tuberculosis. To date, the dosage used is based on studies done in adults and we do not have reliable data for children.

What will happen to my child and what will I have to do if my child is in this study?

If you decide to allow your child to take part in this study a number of investigations will be done. The absorption of the medicines will be studied by placing a needle in a vein. This needle will then be removed and a soft plastic tube will be left in your

child's arm for 8 hours. A small amount of blood will be taken from this tube a total of 5 times in eight hours. No further pricking is required during this period. After 8 hours, the plastic tube will be removed. The next morning, another single blood sample will be taken. The amount of the medicine in the blood will then be measured. All of the above investigations (all 6 blood draws in total) will mean taking less than 2 teaspoonfuls (less than 10ml) of blood from your child.

How many children will take part in the study?

About 30 children will be enrolled in the study.

How long will your child be in the study?

The investigations on your child will be completed as soon after being admitted to Brooklyn Hospital or Tygerberg Children's Hospital as is possible and the examination of your child by the doctor and the blood investigations should be completed within 24 hours.

Why might the doctor not admit my child / baby to this study?

The study doctor may decide that your child is too sick to take part in the study. The study might also be cancelled by the site's Ethics Committee (the committee that watches over the safety and rights of research subjects).

What are the risks of the study?

Adverse reactions to second-line medicines needed to treat drug-resitant TB medicine can occur and your child will be carefully observed for these. Your child/baby's participation in this study will not increase the risk that your child might be affected by any of the tuberculosis medicines, but will increase the chance that such reactions will be detected early.

There is a risk of pain and bleeding at the site of insertion of the IV cannula (blood drawing needle), but this will soon heal. A gentle application of pressure for a few minutes may be required for bleeding after the cannula has been removed.

Are there benefits to taking part in this study?

It is likely that your child/baby may derive no benefit from being in this study. Information from this study may, however, help experts decide what dosage of Terizidone and PAS should be recommended for use in other children in future. The study will therefore only show benefit at a leter stage.

What other choices does my child/baby have besides this study?

Should you decide that you do not want your child/baby to take part in this study your child's/baby's treatment will be unaffected and will continue as usual. Please talk to your doctor about the study if you should be uncertain as to whether your child should take part in the study.

What about confidentiality?

Every effort will be made to keep your child's/baby's personal information confidential, however we cannot guarantee absolute confidentiality. Your child's/baby's personal information may be disclosed if required by law. On our study record we will use codes instead of your child's/baby's name. Only the study staff will know these codes. The study workers will not give out any information about your child/baby without written consent from you. Your family's privacy will be respected. The public health authorities will be notified as usual if your child/baby has TB. Any publication of this study will not use your child's/baby's name or identify your child/baby personally.

Your child's/baby's study records may be inspected by the South African Department of Health, study staff or study monitors. The study records will be kept separate from the normal medical records.

What are the costs to me?

There is no extra cost to you resulting from your child's/baby's participation in the study.

Will I receive any payment?

We will re-imburse you transportation money to enable you to attend the hospital should you wish to be with your child on the day that the study is undertaken.

What happens if my child/baby is injured?

If your child/baby is injured as a result of being in this study, your child/baby will be given immediate treatment for his/her injuries at no cost to you.

What are my rights and my child's rights as a research subject?

Participation in this study is completely voluntary. You may choose not to allow your child/baby to take part in this study or take your child/baby out of the study at any

time. Your child/baby will be treated the same no matter what you decide, there will be no penalty.

We will tell you about new information from this or other studies that may affect your child's/baby's health, welfare or willingness to stay in this study.

What do I do if I have questions or problems?

For questions about this study or a research-related injury, contact: **Prof Bernd Rosenkranz** with above address or the person who gave you this form.

You can contact the committee for Human Research at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed by your child's study doctor.

You will receive a copy of this information and consent form for your own records.

Declaration by parent/legal guardian

By signing below, I *(name of parent/legal guardian)* ……..……. agree to allow my child (name of child) ……..… who is ……. years old, to take part in a research study entitled: Pharmacokinetics and dosing rationale of terizidone and/or PAS in Children.

I declare that:

- I have read or had read to me this information and consent form and that it is written in a language with which I am fluent and comfortable.
- If my child is older than 7 years, he/she must agree to take part in the study and his/her ASSENT must be recorded on this form.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to let my child take part.
- I may choose to withdraw my child from the study at any time and my child will not be penalised or prejudiced in any way.

- My child may be asked to leave the study before it has finished if the study doctor or researcher feels it is in my child's best interests, or if my child does not follow the study plan as agreed to.

Signed at (*place*) .....…..…….. on (*date*) ……...……..

....

Signature of parent/legal guardian **Signature of witness**

Declaration by investigator

I *(name)* ……..…… declare that:

- I explained the information in this document to ……..
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understand all aspects of the research, as discussed above
- I did/did not use an interpreter (i*f an interpreter is used, then the interpreter must sign the declaration below).*

Signed at (*place*) .....…..…….. On (*date*) ……...……..

....

Signature of investigator **Signature of witness**

Declaration by interpreter

I *(name)* ……..…… declare that:

- I assisted the investigator (*name*) ……. to explain the information in this document to (*name of parent/legal guardian*) ……..…….. using the language medium of Afrikaans/Xhosa.
- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the parent/legal guardian fully understands the content of this informed consent document and has had all his/her questions satisfactorily answered.

Signed at (*place*) .....…..…….. On (*date*) ……...……..

…….. ……..

Signature of interpreter **Signature of witness**

Title; Pharmacokinetics and dosing rationale of terizidone and para-aminosalicylic acid (PAS) in children and the evaluation of the in vitro metabolism of ethionamide, terizidone and PAS.

We want to ask you to take part in a study about one of the tablets (terizidone or PAS) that you are taking as part of your TB treatment. In the study we measure the amount of this tablet that goes into your blood and in this way we can see whether we are giving too much, too little or just enough of this specific medicine. To do this, we have to draw a small amount of blood from you. We will apply some ointment to your arm so that the needle prick will be less painful.

A few minutes later we will use a needle to put a soft plastic tube in your arm. The needle will then be taken out and the plastic tube will stay in.
From this plastic tube we will then take small amounts of blood 5 times in a few hours. We only have to prick you once in this time because we can take blood from the soft tube without pricking you again. We will then measure the amount of the medicine in your blood. The soft plastic tube will be taken out. The next morning we will take one more little bit of blood.
You can decide if you want to be part the study or not. You do not have to take part if you don't want to. You can decide at any time to stop being a part of the study if you are unhappy.
Assent of minor
I (*Name of Child/Minor*)……. have been invited to take part in the above research project.

- The study doctor/nurse and my parents have explained the details of the study to me and I understand what they have said to me.
- They have also explained that this study will involve.
- I also know that I am free to withdraw from the study at any time if I am unhappy.
- By writing my name below, I voluntary agree to take part in this research project. I confirm that I have not been forced either by my parents or doctor to take part.

....
Name of child Independent witness

(To be written by the child if possible)

PARTICIPANT INFORMATION AND INFORMED CONSENT FORM FOR RESEARCH INVOLVING GENETIC STUDIES

TITLE OF RESEARCH PROJECT: "Pharmacokinetics and dosing rationale of terizidone and para-aminosalicyclic acid (PAS) in children and in-vitro metabolism of ethionamide, terizidone and PAS."

REFERENCE NUMBER: N09/08/212

PRINCIPAL INVESTIGATOR: Prof Bernd Rosenkranz

ADDRESS: Department of Pharmacology
7th Floor, Clinical Building
Stellenbosch University
Tygerberg Campus
7505

CONTACT NUMBER: 021 938 9331/ 0829550017

We would like to invite your child to participate in a research study that involves genetic analysis and possible long-term storage of blood or tissue specimens. Please take some time to read the information presented here which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how your child could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect your child negatively in any way whatsoever. You are also free to withdraw your child from the study at any point, even if you do agree to take part initially.

If you do not wish your child to participate in this genetic analysis part of the study, they can still be part of the main study of antituberculosis drug levels in the blood. The genetic analysis will then not be performed on your child's blood.

This research study has been approved by the ethics **Committee for Human Research at Stellenbosch University** and it will be conducted according to international and locally accepted ethical guidelines for research, namely the Declaration of Helsinki, and the SA Department of Health's 2004 Guidelines: *Ethics*

in Health Research: Principles, Structures and Processes.

What is Genetic research?

Genetic material, also called DNA or RNA, is usually obtained from a small blood sample. Genes are found in every cell in the human body. Our genes determine what we look like and sometimes what kind of diseases we may be susceptible to. Worldwide, researchers in the field of genetics are continuously discovering new information that may be of great benefit to future generations and also that may benefit people today, who suffer from particular diseases or conditions.

What does this particular research study involve?

In the main part of the study in which your child is involved, the amount of antituberculosis drug in the blood will be measured at various time points. A number of factors can affect the amount of drug in a patient's blood. One of these is the ability of the liver to produce enzymes (substances which speed up chemical reactions). These enzymes are responsible for the metabolism (breakdown) of drugs which are then passed out of the body.

Some people produce more powerful enzymes than others and they are therefore able to break down drugs more quickly than other people. This is because different people have differences in the genes which control how these enzymes are made. These variations in the genetic material of individuals are known as polymorphisms.
This could possibly help us in the future to predict which children are likely to develop very high (which may be dangerous) or very low (the drug doesn't work) concentrations, based on their genetic makeup. If we know their genetic makeup, we could perhaps take precautions or use a lower or higher dose of these drugs.

Why has your child been invited to participate?

- Your child has been invited to participate because you have shown interest in the main part of our study and so we are going to measure blood concentrations of antituberculous drugs in your child. Performing genetic testing on your child will therefore help us determine if genetic differences (polymorphisms) can lead to high or low blood concentrations as explained above.

What procedures will be involved in this research?

Your child will not be required to give any additional blood or participate in additional procedures. The blood obtained from the main study will be used to perform genetic testing.

Are there any risks involved in genetic research?

In this study, we will be focusing on those genes which are responsible for the production of enzymes which break down or metabolize the drugs your child is receiving or drugs which broken down by the same kind of enzymes.

This study therefore will not identify any diseases your child may develop or genetic problems which may lead to diseases later in life.

Are there any benefits to your taking part in this study and will you get told your results?

Your child will not benefit directly from taking part in this genetic research study. You will not be informed of the results. Blood will be stored and only tested at a later date once more is known about the enzymes responsible for drug breakdown and the genetic polymorphisms possibly involved.

It is possible that in the future we can use the study results to predict which children are likely to develop very high (which may be dangerous) or very low (the drug doesn't work) concentrations. If we know their genetic composition, we could perhaps take precautions or use a lower or higher dose of these drugs.

How long will your blood be stored and where will it be stored?

The blood will be stored in the Division of Clinical Pharmacology and analysed by the University of Stellenbosch.

If your blood is to be stored is there a chance that it will be used for other research?

Your blood will only be used for genetic research that is directly related to the metabolism of antituberculosis drugs. Also if the researchers wish to use your stored blood for additional research in this field they will be required to apply for permission to do so from the Human Research Ethics Committee at Stellenbosch University.

If you do not wish your blood specimen to be stored after this research study is

completed you will have an opportunity to request that it be discarded when you sign the consent form.

How will your confidentiality be protected?

The blood for DNA analysis will be labelled with your child's participant number and not name, prior to storage. It will be labelled because we wish to compare your child's blood concentration to their genetic results and so these must be linked by a participant number.

All study records and samples will be kept confidentially in a sealed and locked area only accessible to the investigators. No one other than the investigators or the ethics committee of Stellenbosch University will have access to your child's personal information. In the publication of results, your child's name or identifying information will not be used

Will you or the researchers benefit financially from this research?

You will not be paid to take part in this study.

Important information: In the unlikely event that this research leads to the development of a commercial application or patent, you or your family will not receive any profits or royalties

Declaration by participant

By signing below, I …….…….. agree to take part in a genetic research study entitled *(insert title of study)*.

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is voluntary and I have not been pressurised to take part.
- I have received a signed duplicate copy of this consent form for my records.

Tick the option you choose:

☐ I agree that my blood or tissue sample can be stored **indefinitely/ stored for, ……years,** but I can choose to request at any time that my stored sample be destroyed. My sample will be identified with a special study code that will remain linked to my name and contact details. I have the right to receive confirmation that my request has been carried out. *(NB This option can be excluded completely if the genetic research has no clinical relevance to the patient and you plan to completely and permanently anonymise all samples)*

OR

☐ Please destroy my blood sample as soon as the current research project has been completed.

Signed at (*place*) ..... on (*date*) .....

....

Signature of participant Signature of witness

Declaration by investigator

I *(name)* …… declare that:

- I explained the information in this document to ……..……...
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research as discussed above.
- I did/did not use an interpreter. (*If an interpreter is used then the interpreter must sign the declaration below.*

Signed at (*place*) .....…….. on (*date*) ……...…….. 2005.

....

Signature of investigator Signature of witness

Declaration by Interpreter

I *(name)* …… declare that:

- I assisted the investigator (*name*) …….. to explain the information in this document to (*name of participant*) …….. using the language medium of Afrikaans/Xhosa.
- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.

- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

Signed at (*place*) .....…..…….. on (*date*) ……...……..
2005.

.....	.....
Signature of interpreter	Signature of witness

PARTICIPANT INFORMATION AND INFORMED CONSENT FORM FOR RESEARCH

Title of research project: Pharmacokinetics and dosing rationale of terizidone and para-aminosalicylic acid (PAS) in children and the evaluation of the in vitro metabolism of ethionamide, terizidone and PAS.

Principal Investigator: Prof Bernd Rosenkranz

Address: Faculty of Health Sciences, Stellenbosch University, Francie van Zijl Drive, Tygerberg, 7505

Contact Numbers: Tel +27-21-938 9331 (Office); Cellular phone: +27-82-9550017

You are invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how your child could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do initially agree to take part.

This study has been approved by the **Committee for Human Research at Stellenbosch University** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

Introduction

Tuberculosis (TB) is a very important health problem in South Africa and is often complicated by disseminated forms of TB such as meningitis or widespread lesions (miliary) TB, and resistance to the commonly used medicines. PAS, a second-line medicine is mostly used in combination with the commonly used drugs for the

treatment of extensively resistant TB. When resistance is present other medicines (second-line medicines) must be used. These are not as effective as the medicines usually given for TB and they have more complications than the commonly used (first-line) medicines. The medicines used to treat extensively resistant TB almost always include PAS. The second-line drugs such as PAS are not as effective as first-line TB drugs and it is therefore important that PAS be given in the best possible dosage. There is only very little information available regarding the amount of PAS that gets into the blood. Such information would make it possible for doctors to give much more accurate dosages of PAS than is possible at present. This study aims to determine the amount of PAS that gets into the blood of TB patients following the currently recommended and used dose of PAS.

Before you decide if you want to be a part of this study, we want you to know about the study. You may choose not to be part of this study. No health care will be withheld for you should you choose not to be part of the study. You will still receive the standard treatment for drug-resistant tuberculosis at this hospital.

This is a consent form. It gives you information about this study. The study staff will talk with you about this information. You are free to ask questions about this study at any time. If you agree to take part in this study, you will be asked to sign this consent form. You will get a copy to keep.

Why is this study being done?

The purpose of this study is to evaluate the absorption of the antituberculosis medicine PAS when given in the currently recommended dose in patients with drug-resistant TB.

What will happen to me and what will I have to do if I participate in this study?

If you decide to take part in this study, a number of investigations will be done. The absorption of the medicines will be studied by placing a needle in a vein and taking a small amount of blood 10 times soon after the medicines have been given. The amount of the medicine in the blood will then be measured. All of the above investigations will mean taking the volume of about 2 teaspoons of blood from you at two occasions approximately a month apart.

How many patients will take part in the study?

Up to 40 patients will be enrolled in the study.

How long will you be in the study?

The investigations will be completed as soon after being admitted to Brooklyn Chest Hospital as possible and the examination by the doctor and the blood investigations should be completed within a day and will be repeated about a month later.

Why might the doctor not admit me to this study?

The study doctor may decide that you are too sick to take part in the study. The study might also be cancelled by the site's Ethics Committee (the committee that watches over the safety and rights of research subjects).

What are the risks of the study?

Your participation in this study will not increase the risk that you might be affected by PAS, but will increase the chance that such reactions will be detected early. A small amount of bleeding may occur at the site where the blood is taken from and this may leave a bruise which should soon heal.

Are there benefits to taking part in this study?

It is likely that you may derive no benefit from being in this study. Information from this study may, however, will help experts decide what dosage of PAS should be recommended for use.

What other choices do I have besides this study?

Should you decide that you do not want to take part in this study your treatment will be unaffected and will continue as usual. Please talk to your doctor about the study if you should be uncertain as to whether you should take part in the study.

What about confidentiality?

Every effort will be made to keep your personal information confidential, however we cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. On our study record we will use codes instead of your

name. Only the study staff will know these codes. The study workers will not give out any information about you without written consent from you. Your family's privacy will be respected. The public health authorities will be notified as usual if you have TB. Any publication of this study will not use your name or identify you personally.

Your study records may be inspected by the South African Department of Health, study staff or study monitors. The study records will be kept separate from the normal medical records.

What are the costs to me?

There is no cost to you resulting from participation in the study. All medical care will be provided by the study doctor.

Will I receive any payment?

We will re-imburse your transportation money to enable you to attend the hospital on the day that the study.

What happens if I get injured?

If you get injured as a result of being in this study, you will be given immediate treatment for your injuries at no cost to you.

What are my rights as a research subject?

Participation in this study is completely voluntary. You may choose not to take part in this study or withdraw from the study at any time. You will be treated the same no matter what you decide, there will be no penalty.

We will tell you about new information from this or other studies that may affect your health, welfare or willingness to stay in this study. If you want the results of the study inform the study staff.

What do I do if I have questions or problems?

For questions about this study or a research-related injury, contact either **Prof Bernd Rosenkranz** at the above address or the person who gave you this form.

You can contact the Committee for Human Research at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed by your child's study doctor.

You will receive a copy of this information and consent form for your own records.

Declaration by patient

By signing below, I ……..…… agree to take part in a research study entitled: **Pharmacokinetics and dosing rationale of terizidone and para-aminosalicylic acid (PAS) in children and the evaluation of the in vitro metabolism of ethionamide, terizidone and PAS.**
I declare that:

- I have read or had read to me this information and consent form and that it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to withdraw from the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan as agreed to.

Signed at (*place*) .....…..…….. On (*date*) ……...……..

.....	.....
Signature of parent/legal guardian	Signature of witness

Declaration by investigator

I *(name)* ……..…… declare that:

- I explained the information in this document to ……..
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understand all aspects of the research, as discussed above
- I did/did not use an interpreter (i*f an interpreter is used, then the interpreter must sign the declaration below).*

Signed at (*place*) .....…..…….. On (*date*) ……...……..

..... **.....**

Signature of investigator Signature of witness

Declaration by interpreter

I *(name)* ……..…… declare that:

- I assisted the investigator (*name*) ……. to explain the information in this document to (*name of parent/legal guardian*) ……..…….. using the language medium of Afrikaans/Xhosa.
- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.

- I am satisfied that the parent/legal guardian fully understands the content of this informed consent document and has had all his/her questions satisfactorily answered.

Signed at (*place*) .....…..……. On (*date*) ……...……..

....

Signature of interpreter..... Signature of witness

PARTICIPANT INFORMATION AND INFORMED CONSENT FORM FOR RESEARCH INVOLVING GENETIC STUDIES

TITLE OF RESEARCH PROJECT: Pharmacokinetics and dosing rationale of terizidone and para-aminosalicylic acid (PAS) in children and the evaluation of the in vitro metabolism of ethionamide, terizidone and PAS.

PRINCIPAL INVESTIGATOR: Prof Bernd Rosenkranz

ADDRESS: Faculty of Health Sciences, Stellenbosch University, Francie van Zijl Drive, Tygerberg, 7505

CONTACT NUMBER: Tel +27-21-938 9331 (**Office**); **Cellular phone**: +27-82-9550017

We would like to invite you to participate in a research study that involves genetic analysis and possible long-term storage of blood or tissue specimens. Please take some time to read the information presented here which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how your child could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect your child negatively in any way whatsoever. You are also free to withdraw your child from the study at any point, even if you do agree to take part initially.

If you do not wish to participate in this genetic analysis part of the study, they can still be part of the main study of antituberculosis drug levels in the blood. The genetic analysis will then not be performed on your blood.

This research study has been approved by the ethics **Committee for Human Research at Stellenbosch University** and it will be conducted according to international and locally accepted ethical guidelines for research, namely the Declaration of Helsinki, and the SA Department of Health's 2004 Guidelines: *Ethics*

in Health Research: Principles, Structures and Processes.

What is Genetic research?

Genetic material, also called DNA or RNA, is usually obtained from a small blood sample. Genes are found in every cell in the human body. Our genes determine what we look like and sometimes what kind of diseases we may be susceptible to. Worldwide, researchers in the field of genetics are continuously discovering new information that may be of great benefit to future generations and also that may benefit people today, who suffer from particular diseases or conditions.

What does this particular research study involve?

In the main part of the study the amount of antituberculosis drug in the blood will be measured at various time points. A number of factors can affect the amount of drug in a patient's blood. One of these is the ability of the liver to produce enzymes (substances which speed up chemical reactions). These enzymes are responsible for the metabolism (breakdown) of drugs which are then passed out of the body.

Some people produce more powerful enzymes than others and they are therefore able to break down drugs more quickly than other people. This is because different people have differences in the genes which control how these enzymes are made. These variations in the genetic material of individuals are known as polymorphisms

This could possibly help us in the future to predict which patients are likely to develop very high (which may be dangerous) or very low (the drug doesn't work) concentrations, based on their genetic makeup. If we know your genetic makeup, we could perhaps take precautions or use a lower or higher dose of these drugs.

Why have I been invited to participate?

You have been invited to participate because you have shown interest in the main part of our study and so we are going to measure blood concentrations of antituberculous drugs in you. Performing genetic testing on will therefore help us determine if genetic differences (polymorphisms) can lead to high or low blood concentrations as explained above.

What procedures will be involved in this research?

You will not be required to give any additional blood or participate in additional procedures. The blood obtained from the main study will be used to perform genetic testing.

Are there any risks involved in genetic research?

In this study, we will be focusing on those genes which are responsible for the production of enzymes which break down or metabolize the drugs your child is receiving or drugs which broken down by the same kind of enzymes.

This study therefore will not identify any diseases or genetic problems which may lead to diseases later in life.

Are there any benefits to your taking part in this study and will you get told your results?

You will not benefit directly from taking part in this genetic research study. You will not be informed of the results. Blood will be stored and only tested at a later date once more is known about the enzymes responsible for drug breakdown and the genetic polymorphisms possibly involved.

It is possible that in the future we can use the study results to predict which patients are likely to develop very high (which may be dangerous) or very low (the drug doesn't work) concentrations. If we know your genetic composition, we could perhaps take precautions or use a lower or higher dose of these drugs.

How long will your blood be stored and where will it be stored?

The blood will be stored in the Division of Clinical Pharmacology and analysed by the University of Stellenbosch.

If your blood is to be stored is there a chance that it will be used for other research?

Your blood will only be used for genetic research that is directly related to the metabolism of antituberculosis drug PAS. If you do not wish your blood specimen to be stored after this research study is completed you will have an opportunity to request that it be discarded when you sign the consent form.

How will your confidentiality be protected?

The blood for DNA analysis will be labelled with your participation number and not name, prior to storage. It will be labelled because we wish to compare your child's blood concentration to their genetic results and so these must be linked by a participant number.

All study records and samples will be kept confidentially in a sealed and locked area only accessible to the investigators. No one other than the investigators or the ethics committee of Stellenbosch University will have access to your child's personal information. In the publication of results, your child's name or identifying information will not be used

Will you or the researchers benefit financially from this research?

You will not be paid to take part in this study.

Important information: In the unlikely event that this research leads to the development of a commercial application or patent, you or your family will not receive any profits or royalties.

Declaration by participant

By signing below, I ……..……. agree to take part in a genetic research study entitled *(insert title of study)*.

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is voluntary and I have not been pressurised to take part.
- I have received a signed duplicate copy of this consent form for my records.

Tick the option you choose:

☐ I agree that my blood sample can be stored **indefinitely/ stored for ……years,** but I can choose to request at any time that my stored sample be destroyed. My sample will be identified with a special study code that will remain linked to my name and contact details. I have the right to receive confirmation that my request has been carried out

OR

☐ Please destroy my blood sample as soon as the current research project has been completed.

Signed at (*place*) ..... on (*date*) .....

Signature of participant **Signature of witness**

Declaration by investigator

I *(name)* …… declare that:

- I explained the information in this document to ……..……...
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research as discussed above.
- I did/did not use an interpreter. (*If an interpreter is used then the interpreter must sign the declaration below.*

Signed at (*place*) .....…..…….. on (*date*)
……...20.....

....

Signature of investigator **Signature of witness**

Declaration by Interpreter

I *(name)* …… declare that:

- I assisted the investigator (*name*) …….. to explain the information in this document to (*name of participant*) …….. using the language medium of Afrikaans/Xhosa.
- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.

- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

Signed at (*place*) .....…….....…….. On (*date*) ……..... 20.....

....

Signature of interpreter **Signature of a witness**

Appendix E: CRF of Study 1

STELLENBOSCH UNIVERSITY
Case record form

Title of the project: Pharmacokinetics and dosing rationale of terizidone and para-aminosalicylic acid (PAS) in children and the evaluation of the in vitro metabolism of ethionamide, terizidone and PAS.

Project No……
Date……..
Patient name……
Date of birth…….
Sex……..
Race…….
Diagnosis……
……..

Measurements:

Height (cm)…….
Weight (kg)……..
Mid upper arm circumference (cm)……..
Drug for measurement (name)…….Dose (mg)…….
Dosage time……

Sample	**Sampling time (min)**		**Time (received)**	**Remarks**
	Planned	Actual sampling		
1				
2				
3				
4				
5				
6				

Details of the co-administered drugs

Drug (name)	Dose(mg)	Dosage time	Duration of Rx

Appendix F: Informed consent of Study 2

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

TITLE OF THE RESEARCH PROJECT:

Pharmacokinetics of twice daily vs. once daily dosing with granular slow-release para-aminosalicylic acid (PAS) in adults on second-line anti-tuberculosis and antiretroviral treatment

REFERENCE NUMBER:

PRINCIPAL INVESTIGATOR:

Lizanne de Kock

ADDRESS:

Division of Pharmacology
Department of Medicine
University of Stellenbosch
PO Box 19063
Tygerberg, Cape Town, 7505
South Africa

CONTACT NUMBER:

Phone: 021 9389336
Fax: 021-932 6958
E-mail: 15180271@sun.ac.za

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Health Research Ethics Committee at Stellenbosch University** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

Why have you been invited to participate?

- You have been invited to take part in this study because you meet the requirements for the study i.e. adult, M/XDR-TB etc.

What is this research study all about?

- Forty eight (48) adults will sign up for the study at Brooklyn Hospital for Chest Diseases and/or D.P. Marais Hospital, Cape Town, South Africa.
- PAS is known to cause nausea, bloating, diarrhoea, and abdominal pain; and with the use of multiple medicines, we want to see if it is possible to decrease the daily dosing of PAS from twice daily to once daily. In addition we want to look at possible interactions of PAS and HIV/AIDS treatment.
- *Procedure*

  You will take part in the study for three weeks. You will receive PAS once daily or twice daily, depending on your group. You will follow the prescribed dosing for eight (8) days. On day 8 a blood samples will be taken before you will receive your prescribed medication. After that, three millilitre (3ml) blood samples will be taken at 10 time points during the day. Thirty millilitres (30ml) of blood will be taken at every sampling day. For the following 8 days you will

follow the once-daily dosing if you were on the twice-daily and visa versa (cross-over), before blood samples will be taken again. You will still be on your other prescribed medicines, which will be recorded. Only the PAS dosing will change. Each day you will be asked to fill in a self-rating form, where you must rate discomfort in your stomach, nausea, a feeling of fullness or any other symptoms you may be experiencing.

What will your responsibilities be?

- To co-operate with the research nurse and personal involved in the study. To complete the self-rating forms each day.

Will you benefit from taking part in this research?

- You and future patients may potentially benefit from the once daily dosing, in that less frequent dosing is needed. The study will provide important valuable information for the optimal dose regimen.

Are there in risks involved in your taking part in this research?

You will be treated as usual by the attending doctor, you will be already on PAS before enrolment and your treatment will not change, except for whether you take PAS once a day or twice a day. You may experience side effects by using PAS such as nausea, abdominal pain etc. and will be treated accordingly. There may be a risk that you will experience pain, bruising and/or bleeding at the side of 20-gauge angiocatheter insertion. In addition, there is a risk that you may experience unexpected side-affects and that the once-daily dosage may not be as effective as the standard dosage. However, you will be monitored for side- affect by your attending nurse or doctor. For any trial related emergency please contact Dr AH Diacon at 021 938 9392 or 082 420 1677

Who will have access to your medical records?

- The research nurse and study personal will have access to your information. The information collected and results obtained will be treated as confidential

and protected by using a subject numbers. If this information is used in a publication or thesis, your identity will remain anonymous.

Will you be paid to take part in this study and are there any costs involved?

You will be compensation for inconvenience. There will be no costs involved for you, if you do take part. You will receive R 150 per sampling day.

Is there anything else that you should know or do?

- **You can contact Dr AH Diacon at tell 021 938 9392 or 082 420 1677 if you have any further queries or encounter any problems.**
- You can contact the Health Research Ethics Committee at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed by your study doctor.
- You will receive a copy of this information and consent form for your own records.

Declaration by participant

By signing below, I ……..……. agree to take part in a research study entitled *(insert title of study)*.

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.

- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (*place*) .....…..…….. on (*date*) ……...…….. 2012.

....

Signature of participant **Signature of witness**

Declaration by investigator

I *(name)* ……..…… declare that:

- I explained the information in this document to ……..
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use an interpreter. (*If an interpreter is used then the interpreter must sign the declaration below.*

Signed at (*place*) .....…..…….. on (*date*) ……...…….. 2012.

....

Signature of investigator **Signature of witness**

Declaration by interpreter

I *(name)* ……..…… declare that:

- I assisted the investigator (*name*) ……. to explain the information in this document to (*name of participant*) ……..…….. using the language medium of Afrikaans/Xhosa.
- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

Signed at (*place*) .....…….. on (*date*) ……...……..

....

Signature of interpreter **Signature of witness**

PARTICIPANT INFORMATION AND INFORMED CONSENT FORM FOR RESEARCH INVOLVING GENETIC STUDIES

TITLE OF RESEARCH PROJECT:

Pharmacokinetics of twice daily vs. once daily dosing with granular slow-release para-aminosalicylic acid (PAS) in adults on second-line anti-tuberculosis and antiretroviral treatment

REFERENCE NUMBER:

PRINCIPAL INVESTIGATOR:

Lizanne de Kock

ADDRESS:

Division of Pharmacology
Department of Medicine
University of Stellenbosch
PO Box 19063
Tygerberg, Cape Town, 7505
South Africa

CONTACT NUMBER:

Phone: 021 9389336
Fax: 021-932 6958
E-mail: 15180271@sun.ac.za

We would like to invite you to participate in a research study that involves genetic analysis and possible long-term storage of blood or tissue specimens. Please take some time to read the information presented here which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to

participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part initially.

This research study has been approved by the ethics **Health Research Ethics Committee at Stellenbosch University** and it will be conducted according to international and locally accepted ethical guidelines for research, namely the Declaration of Helsinki, and the SA Department of Health's 2004 Guidelines: *Ethics in Health Research: Principles, Structures and Processes.*

What is Genetic research?

Genetic material, also called DNA or RNA, is usually obtained from a small blood sample. Occasionally genetic material is obtained from other sources such as saliva or biopsy specimens. (A biopsy is a tiny piece of tissue that is cut out e.g. from the skin or from a lump, to help your doctor make a diagnosis.) Genes are found in every cell in the human body. Our genes determine what we look like and sometimes what kind of diseases we may be susceptible to. Worldwide, researchers in the field of genetics are continuously discovering new information that may be of great benefit to future generations and also that may benefit people today, who suffer from particular diseases or conditions.

Why have you been invited to participate?

You have been invited to take part in this study because you meet the requirements of the study i.e. adult, M/XDR-TB, etc.

What does this particular research study involve?

- PAS is known to cause nausea, bloating, diarrhoea, and abdominal pain; and with the use of multiple medicines, we want to compare once daily dosing of PAS with twice daily. In addition we want to look at possible interactions between PAS and HIV/AIDS treatment. Variations in the genes may influence your response to certain medicines. We hope to find the specific variations which influence how your body response to the medicine.

What procedures will be involved in this research?

- No additional blood is required for genotyping

Are there any risks involved in genetic research?

- There are no risks related to the study.

Are there any benefits to your taking part in this study and will you get told your results?

*Your personal results will be made known to you **only if they indicate** that you may:*

- *Have a definite risk for developing a particular disorder.*
- *Have a condition or predisposition to developing a condition that is treatable or avoidable e.g. by a lifestyle modification.*
- *Need genetic counselling.*

How long will your blood be stored and where will it be stored?

The sample will be stored for 2 years depending on the results of the study.
Your specimen will be stored at the University of Stellenbosch Medical School in a dedicated fridge or freezer for at least 2 years or at the laboratory that performed the same test(s) as a routine service.

If your blood is to be stored is there a chance that it will be used for other research?

Your specimen will only be used for the genetic research as indicated above and any further testing will only be done after obtaining full written consent. Also, if the researchers wish to use your stored specimen for **additional research in this field** they will be required to apply for permission to do so from the Human Research Ethics Committee at Stellenbosch University (that can be contacted at telephone number 021 938 9657).

If you do not wish your blood specimen to be stored after this research study is

completed you will have an opportunity to request that it be discarded when you sign the consent form.

How will your confidentiality be protected?

The specimens will be given a subject number and only the researchers, clinicians and laboratory personnel involved in the study will have access to the self-rating scales and consent forms. Specimens sent to other laboratories locally or abroad will be shipped only with the subject and sample number attached to them and your name will not be disclosed to the collaborator. If ever information comes to light that could be important to you or your descendants, all possible attempts will be made to contact you and your counsel. The results of the study will be presented at congresses/workshops/ professional forums and included in scientific articles and student theses, without revealing the identity of the study participants.

Will you or the researchers benefit financially from this research?

- You will be compensation for inconvenience. There will be no costs involved for you, if you do take part.
- The researchers will not financial benefit from the study, but there may be indirect financial benefits such as publications etc. to the investigators.

Important information: In the unlikely event that this research leads to the development of a commercial application or patent, you or your family will not receive any profits or royalties

Declaration by participant

By signing below, I ……..…….. agree to take part in a genetic research study entitled *(insert title of study)*.

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is voluntary and I have not been pressurised to take part.
- I have received a signed duplicate copy of this consent form for my records.

Tick the option you choose:

☐ I agree that my blood or tissue sample can be stored **indefinitely/ stored for, ……years,** but I can choose to request at any time that my stored sample be destroyed. My sample will be identified with a special study code that will remain linked to my name and contact details. I have the right to receive confirmation that my request has been carried out. *(NB This option can be excluded completely if the genetic research has no clinical relevance to the patient and you plan to completely and permanently anonymise all samples)*

OR

☐ I agree that my blood or tissue sample can be stored **indefinitely/ stored for,……years** after the project is completed but that it is anonymised with all possible links to my identity removed, and that the researchers may then use it for additional research in this or a related field. Once my sample is anonymised, my rights to that sample are waivered. My sample may be shipped to another laboratory in SA or abroad to be used in other research projects in this or a related field

OR

☐ Please destroy my blood sample as soon as the current research project has been completed.

Signed at (*place*) ..... on (*date*) .....

....

Signature of participant **Signature of witness**

Declaration by investigator

I *(name)* …… declare that:

- I explained the information in this document to ……..……...
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research as discussed above.
- I did/did not use an interpreter. (*If an interpreter is used then the interpreter must sign the declaration below.*

Signed at (*place*) .....…..…….. On (*date*) ……...…….. 2012.

....

Signature of investigator **Signature of witness**

Declaration by Interpreter

I *(name)* …… declare that:

- I assisted the investigator (*name*) ……. to explain the information in this document to (*name of participant*)

…….. Using the language medium of Afrikaans/Xhosa.

- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

Signed at (*place*) .....…..…….. On (*date*) ……...…….. 2012.

Appendix G: CRF of Study 2

PAS ONCE DAILY VS TWICE DAILY PK STUDY BASELINE RECORDING FORM

M12/01/006

Patient subject

DATE	D	D	M	M	Y	Y	Y	Y

Title of the Project: Pharmacokinetics of twice daily vs. once daily dosing with granular slow-release para-aminosalicylic acid in adults on second-line anti-tuberculosis and antiretroviral treatment

BASELINE MEASUREMENTS
Mark with an X

A	CONSENT
	Explained the study and procedures to the study subject
	Encourage the study subject to asked any questions
	Received VERBAL consent for the study
	Received WRITTEN informed consent for the study
	Patient received a copy of the consent
	Copy of the consent is in patient file

B	GENERAL
Language	
Date of Birth	
Gender	
Race	

C	MEASUREMENTS	
Weight		kg
Height/length		cm

D	DIAGNOSES				
	MDR-TB		Pre- XDR-TB		XDR-TB

E	HIV STATUS		
	Positive		Negative

F	CHEMISTRY	RANGE	DATE	REF RANGE
POTASSIUM				3.3-5.3
CREATININE				64-104
HAEMAGLOBIN				13.0-17.0
THYROID STIMULATING HORMONE				0.49-5.66
ALANINE TRANSAMINASE (ALT)				5-40

G	CONCURRENT ILLNESS	
ILLNESS		**DATE**

H	MEDICATION USED
PLEASE FIND ATTACHED	

	DATE								SIGNATURE
INVESTIGATOR	D	D	M	M	Y	Y	Y	Y	
INVESTIGATOR									

PAS ONCE DAILY VS TWICE DAILY PK STUDY

Case Report form
M12/01/006

Patient subject

PK DATE	D	D	M	M	Y	Y	Y	Y

Title of the Project: Pharmacokinetics of twice daily vs. once daily dosing with granular slow-release para-aminosalicylic acid in adults on second-line anti-tuberculosis and antiretroviral treatment

PK SAMPLING VISIT	1	2

A	STUDY GROUP		
1	1x 8g GSR-PAS	2	2x 4g GSR-PAS

B	GENERAL
Date of Birth	
Gender	
Race	

C	MEASUREMENTS		
1	Weight		kg
2	Height/Length		cm
3	Blood pressure		

D	PAS DOSING					
	PAS DOSING	TIME 1	YOGHURT FLAVOUR	TIME 2	YOGHURT FLAVOUR	COMMENTS
1	1x 8g GSR-PAS					
2	2x 4g GSR-PAS					

PAS ONCE DAILY VS TWICE DAILY PK STUDY

Case Report form

M12/01/006

Patient subject

PK DATE	D	D	M	M	Y	Y	Y	Y

E	PHARMACOKINETIC SAMPLING			
Tubes		Sampling time		COMMENTS
time	Tube name e.g. 1/DI50T1	Planned	Actual	
(t_0)	T0			
DOSE TIME 1				
1	T1			
2	T2			
3	T3			
4	T4			
6	T6			
8	T8			
12	T12			
DOSE TIME 2				
24	T24			

PAS ONCE DAILY VS TWICE DAILY PK STUDY

Case Report form
M12/01/006

Patient subject

PK DATE	D	D	M	M	Y	Y	Y	Y

F	**DRUGS, DOSAGES, FORMULATIONS ON SAMPLING DAY**		
DRUGS	DOSAGE (mg)	DOSING TIME	ADMINISTRATION 1 – IM 2 – Oral
TB			
Amikacin			
Capreomycin			
Clarythromycin			
Dapsone			
Ethambutol			
Ethionamide			
INH			
Kanamycin			
Levofloxacin			
Moxifloxacin			
Ofloxacin			
Pyrazinamide PZA			
Terizidone			

ARVs			
Efavirenz (EFV)			
Stavudine (d4T)			
Lamivudine (3TC)			
Zidovudine (AZT)			
Aluvia			

PAS ONCE DAILY VS TWICE DAILY PK STUDY
Case Report form
M12/01/006

Patient subject

PK DATE	D	D	M	M	Y	Y	Y	Y

DRUGS (mg)	DOSAGE	DOSING TIME	ADMINISTRATION 1 – IM 2– Oral
ANTI-PSYCHOTICS			
Amitriptyline			
Trepilene			
Modecote			
Haloperidol			

OTHER			

PAS ONCE DAILY VS TWICE DAILY PK STUDY

Case Report form
M12/01/006

Patient subject

PK DATE	D	D	M	M	Y	Y	Y	Y

G	MEALS								
MORNING	Time Breakfast given	H	H	m	m				
Mark ✓ if applicable	Porridge with milk and sugar : Oats/Maize meal	Quantity				¼	½	Full	
Mark ✓ if applicable	Sandwich with margarine	Slices of bread							
Mark ✓ if applicable	Other	Specify							
LUNCH									
Mark ✓ if applicable									
Mark ✓ if applicable									
Mark ✓ if applicable									
EVENING									
Mark ✓ if applicable									
Mark ✓ if applicable									
Mark ✓ if applicable									
Mark ✓ if applicable									
Tea									
Mark ✓ if applicable	Rooibos								
Mark ✓ if applicable	Ceylon								
Mark ✓ if applicable	Other	Specify							
Mark ✓ if applicable	Milk								
Mark ✓ if applicable	Juice								
Mark ✓ if applicable	Other Meal	Specify							

H	SIDE-EFFECTS		
1	Was there any significant vomiting/regurgitation/reflux noted?		
	0 - No	1 - Yes	-5 - Unknown
	(Note : If Yes, medications require re-administration as deemed clinically relevant)		

PAS ONCE DAILY VS TWICE DAILY PK STUDY
Case Report form
M12/01/006

Patient subject

COMMENT:

	DATE								SIGNATURE
INVESTIGATOR/NURSE	D	D	M	M	Y	Y	Y	Y	
INVESTIGATOR/NURSE	D	D	M	M	Y	Y	Y	Y	

Appendix H: Dosing form for PAS in Study 2

PAS ONCE DAILY VS TWICE DAILY PK STUDY
Dosing and VAS forms
M12/01/006

Patient subject

PK DATE	D	D	M	M	Y	Y	Y	Y

Title of the Project: Pharmacokinetics of twice daily vs. once daily dosing with granular slow-release para-aminosalicylic acid in adults on second-line anti-tuberculosis and antiretroviral treatment

A	STUDY GROUP		
1	1x 8g GSR-PAS	2	2x 4g GSR-PAS

B	PAS DOSING				
	PAS DOSING	TIME 1		TIME 2	
1	1x 8g GSR-PAS				
2	2x 4g GSR-PAS				

C	TAKE PAS WITH			
	PAS DOSNING	AMOUNT	FLAVOUR	COMMENTS
1	YOGHURT			
2	YOGHURT			

D	COMPLETED VAS FORM		
1	YES	2	NO

	Was there any significant vomiting/regurgitation/reflux noted?		
	0 - No	1 - Yes	-5 - Unknown
	(Note : If Yes, medications require re-administration as deemed clinically relevant)		

	DATE								SIGNATURE
INVESTIGATOR/NURSE	D	D	M	M	Y	Y	Y	Y	
INVESTIGATOR/NURSE	D	D	M	M	Y	Y	Y	Y	

Appendix I: VAS form for Study 2

MDR PK STUDY

0-10 VAS NUMERIC SCALES:
GASTROINTESTINAL AND OTHER INTOLERANCES

Version 2012-01-16

SUBJECT NUMBER

RATING DATE	D	D	M	M	Y	Y	Y	Y

PLEASE COMPLETE THE FOLLOWING SCALE BY PLACING A VERTICAL MARK ON THE LINES BELOW TO INDICATE HOW YOU FEEL TODAY:

0: No side effects
5: Moderate side effects
10: Worst extreme side effects

Did you experience any abdominal pain or cramps today and how will you classify it?

☐ **Morning** ☐ **Mid-Day** ☐ **Evening**

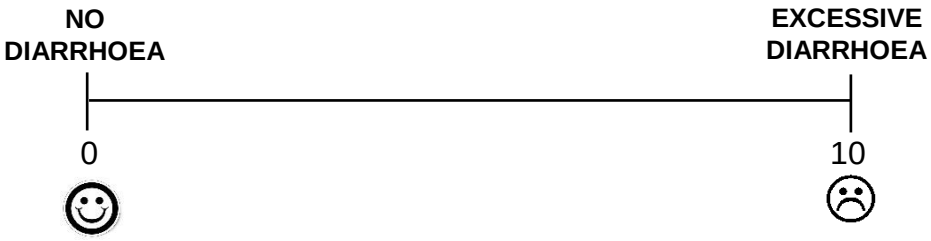
Did you experience nausea today and how will you classify it?

☐ **Morning** ☐ **Mid-Day** ☐ **Evening**

Did you vomit today, if 'Yes' how many times?

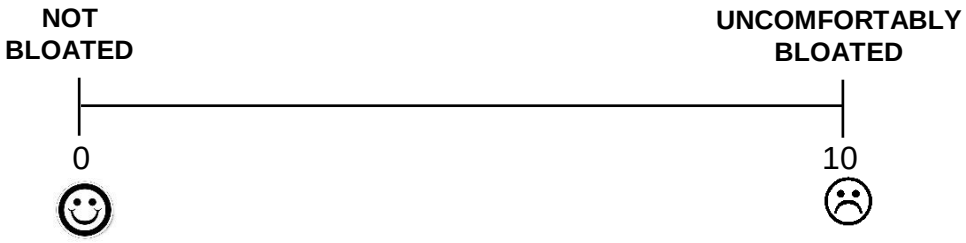
☐ 1 ☐ 2 ☐ 3 ☐ 4-5

Did you experience any diarrhoea today and how will you classify it?



☐ Morning ☐ Mid-Day ☐ Evening

Are/were you bloated today and how will you classify it?



☐ Morning ☐ Mid-Day ☐ Evening

Please comment on any other adverse events

Comments:……

……

……

Investigator/Nurse's signature:……

Appendix J: Individual pharmacokinetic parameters of Study 1

Individual pharmacokinetic parameters for patients administered 4 g twice-daily GSR-PAS on the first occasion

Parameter	PK Parameters of GSR-PAS											
	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
C_{max}, µg/ml	25.8	48.95	38.8	29.05	48.4	93.1	48.95	37.3	87.05	44.65	61.05	42.75
t_{max},h	6.08	5	5	6.08	5	8	5	6.08	5	8	4	0
C_{min}, µg/ml	1.8	8.77	4.05	3.27	4.83	31.05	8.77	12.25	24.95	12.6	16.75	13.5
t_{min},h	12	12	12	0	0	0	12	12	8	3	12	5
C_{last}, µg/ml	1.8	8.77	4.05	7.61	9.1	91.5	8.77	12.25	37.2	15.8	16.75	29.35
t_{last},h	12	12	12	12	12	12	12	12	12	12	12	12
C_0, µg/ml	2.18	31.2	6.99	3.27	4.83	31.05	31.15	27.55	32.2	26.4	31	42.75
C_{12}, µg/ml	1.8	8.77	4.05	7.61	9.1	91.5	8.77	12.25	37.2	15.8	16.75	29.35
C_{24}, µg/ml	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing
AUC_{last}, µg.h/ml	146.97	295.89	242.24	188.81	294.94	852.06	295.84	310.63	570.2	324.35	543.33	290.3
AUC_{12}, µg.h/ml	146.97	295.89	242.24	188.81	294.94	852.06	295.84	310.63	570.2	324.35	543.33	290.3
AUC_{24}, µg.h/ml	150.9	327.82	253.01	220.73	331.56	Missing	327.77	367.83	776.64	Missing	615.25	Missing
AUC_{∞}, µg.h/ml	150.88	331.85	252.99	223.74	333.06	Missing	331.8	372.79	857.52	Missing	619.16	Missing
$t_{1/2}$, h	1.51	2.84	1.84	3.18	2.9	Missing	2.84	3.52	5.35	Missing	3.14	Missing
CL_{ss}/F, L/h	27.22	13.52	16.51	21.19	13.56	4.69	13.52	12.88	7.02	12.33	7.36	13.78
V_z/F_{obs}, L	57.65	49.42	42	82.06	50.32	Missing	49.43	54.44	36.03	Missing	29.25	Missing

Individual pharmacokinetic parameters for patients administered 4 g twice-daily GSR-PAS on the first occasion

Parameter	PK Parameters of GSR-PAS											
	1	2	3	4	5	6	7	8	9	10	11	12
C_{max}, µg/ml	106.5	44.6	Missing	72.15	80.65	32.2	23.15	17.05	119	35.1	13.35	45.45
t_{max},h	6	2.92	Missing	2	6	1	8	0	6	5	2.02	4.18
C_{min}, µg/ml	51.7	16.3	Missing	38.7	6.54	6.27	0.23	0.3	57.8	4.21	2.16	17.55
t_{min},h	2	0	Missing	0	1	6	4	3.05	0	0	8	0
C_{last}, µg/ml	66.1	29.95	Missing	55.9	73.15	18.8	23.15	7.39	89.3	31.6	2.16	30.5
t_{last},h	8	7.92	Missing	8	8	8	8	8	8	8	8	8
C_0, µg/ml	55.55	16.3	Missing	38.7	7.14	27.9	2.68	17.05	57.8	4.21	11	17.55
C_{12}, µg/ml	43.3	8.46	Missing	21.2	56.1	29.15	8.56	5.93	53.55	19.8	0.93	26.2
C_{24}, µg/ml	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing
AUC_{last}, µg.h/ml	557.78	228.44	Missing	459.25	292.1	141.05	63.56	66.82	726.8	211.76	56.38	268.03
AUC_{12}, µg.h/ml	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	61.92	368.69
AUC_{24}, µg.h/ml	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	65.08	507.73
AUC_{∞}, µg.h/ml	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	65.24	562.29
$t_{1/2}$, h	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	2.85	6.69
CL_{ss}/F, L/h	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	68.61	13.46
V_z/F_{obs}, L	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	252.16	68.63

Individual pharmacokinetic parameters for patients administered 4 g twice-daily GSR-PAS on the first occasion

Parameter	PK Parameters of GSR-PAS											
	13	14	15	16	17	18	19	20	21	22	23	24
C_{max}, µg/ml	41.3	38.5	30.2	20.4	51.1	88.35	71.9	77.5	31.95	84.85	56.45	32.35
t_{max},h	0	4	3	4.02	6	4	4	5.03	6	4	4	8
C_{min}, µg/ml	32.85	1.19	1.32	7.87	24.75	44.2	21	27.45	1.49	25.3	2.25	4.23
t_{min},h	8	0	0	0	0	0	0	2	2	0	0	0
C_{last}, µg/ml	32.85	22.3	15.8	11.95	43.8	69.1	56.95	60.1	25.6	47.35	20.45	32.35
t_{last},h	8	8	8	8	8	8	8	8	8	8	8	8
C_0, µg/ml	41.3	1.19	1.32	7.87	24.75	44.2	21	30.2	4.35	25.3	2.25	4.23
C_{12}, µg/ml	51.1	44.85	48.55	30.2	28.4	73.15	36.05	17.75	71.3	17.1	1.55	93.45
C_{24}, µg/ml	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing
AUC_{last}, µg.h/ml	289	192.64	143.92	107.33	331.85	566.42	440.55	461.2	133.93	508.23	276.85	173.15
AUC_{12}, µg.h/ml	Missing	262.17	187.03	143.41	Missing	Missing	Missing	664.37	Missing	653.58	321.65	Missing
AUC_{24}, µg.h/ml	Missing	340.28	232.41	185.25	Missing	Missing	Missing	976.45	Missing	809.65	335.48	Missing
AUC_{∞}, µg.h/ml	Missing	358.99	247.22	197.87	Missing	Missing	Missing	1138.62	Missing	843.86	335.22	Missing
$t_{1/2}$, h	Missing	5.17	4.53	5.25	Missing	Missing	Missing	7.81	Missing	4.91	1.98	Missing
CL_{ss}/F, L/h	Missing	18.72	25.45	33.85	Missing	Missing	Missing	7.71	Missing	7.24	13.58	Missing
V_z/F_{obs}, L	Missing	83.12	105.78	153.17	Missing	Missing	Missing	39.6	Missing	33.6	34.06	Missing

Individual pharmacokinetic parameters for patients administered 4 g twice-daily GSR-PAS on the first occasion

Parameter	PK Parameters of GSR-PAS				
	25	26	27	28	29
C_{max}, µg/ml	39	86.7	53.15	42.8	55.4
t_{max},h	8	5	6	3	6
C_{min}, µg/ml	2.63	35.4	27.2	2.2	22.65
t_{min},h	2	0	2	0	0
C_{last}, µg/ml	39	70.05	45.25	13.25	54.65
t_{last},h	8	8	8	8	8
C_0, µg/ml	5.18	35.4	35.75	2.2	22.65
C_{12}, µg/ml	57.05	44.85	22.3	0.6	53.85
C_{24}, µg/ml	Missing	Missing	Missing	Missing	Missing
AUC_{last}, µg.h/ml	154.9	511.03	344.08	228.3	324.38
AUC_{12}, µg.h/ml	Missing	Missing	Missing	257.27	Missing
AUC_{24}, µg.h/ml	Missing	Missing	Missing	266.04	Missing
AUC_{∞}, µg.h/ml	Missing	Missing	Missing	265.83	Missing
$t_{1/2}$, h	Missing	Missing	Missing	1.96	Missing
CL_{ss}/F, L/h	Missing	Missing	Missing	16.68	Missing
V_z/F_{obs}, L	Missing	Missing	Missing	42.62	Missing

Summary pharmacokinetic parameters for patients administered 4 g twice-daily GSR-PAS on the first occasion

Parameter	PK Parameters of GSR-PAS							
	N	Mean	SD	Min	Median	Max	CV%	Geometric Mean
C_{max}, µg/ml	40	52.42	25.46	13.35	45.05	119	49	46.61
t_{max},h	40	4.66	2.15	0	5	8	46	Missing
C_{min}, µg/ml	40	15.71	15.17	0.23	10.51	57.8	97	8.12
t_{min},h	40	3.2	4.42	0	0.5	12	138	Missing
C_{last}, µg/ml	40	33.29	24.49	1.8	29.65	91.5	74	23.44
t_{last},h	40	9.2	1.86	7.92	8	12	20	9.03
C_0, µg/ml	40	20.89	16.04	1.19	21.83	57.8	77	13.04
C_{12}, µg/ml	40	30.21	24.81	0.6	24.25	93.45	82	18.28
C_{24}, µg/ml	0	Missing	Missing	Missing	Missing	Missing	Missing	Missing
AUC_{last}, µg.h/ml	40	315.38	182.72	56.38	291.2	852.06	58	263.61
AUC_{12}, µg.h/ml	21	346.46	198.98	61.92	295.84	852.06	57	295.71
AUC_{24}, µg.h/ml	18	393.88	247.72	65.08	329.69	976.45	63	326.75
AUC_{∞}, µg.h/ml	18	416.05	281.91	65.24	332.46	1138.62	68	338.58
$t_{1/2}$, h	18	3.79	1.76	1.51	3.16	7.81	46	3.43
CL_{ss}/F, L/h	21	17.57	13.73	4.69	13.56	68.61	78	14.47
V_z/F_{obs}, L	18	70.18	54.89	29.25	49.87	252.16	78	58.43

Individual pharmacokinetic parameters for patients administered 4 g twice-daily GSR-PAS on the second occasion

Parameter	PK Parameters of GSR-PAS											
	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
C_{max}, µg/ml	35.8	32.45	38	40.3	21.05	52.65	29	16.35	69.5	50.6	50.3	15.7
t_{max},h	8	0	4	4	8	8	4	8	5	5	6.08	2
C_{min}, µg/ml	9.86	4.97	6.71	3.6	0.26	1.58	4.84	3.9	25.55	0.31	13.55	1.23
t_{min},h	2	2	0	12	0	2	12	6	3	0	2	8
C_{last}, µg/ml	34	19.8	6.82	3.6	6.63	15.95	4.84	5.4	28.95	2.52	34.3	2.33
t_{last},h	12	12	12	12	12	12	12	12	12	12	12	12
C_0, µg/ml	17.65	32.45	6.71	6.63	0.26	3.33	16.7	14	27.2	0.31	15.4	7.57
C_{12}, µg/ml	34	19.8	6.82	3.6	6.63	15.95	4.84	5.4	28.95	2.52	34.3	2.33
C_{24}, µg/ml	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing
AUC_{last}, µg.h/ml	267.97	164.73	248.55	176.59	103.06	285.93	172.36	109.23	540.21	231.45	407.06	64.97
AUC_{12}, µg.h/ml	267.97	164.73	248.55	176.59	103.06	285.93	172.36	109.23	540.21	231.45	407.06	64.97
AUC_{24}, µg.h/ml	Missing	Missing	274.61	187.49	Missing	Missing	194.22	Missing	706.52	236.59	Missing	73
AUC_{∞}, µg.h/ml	Missing	Missing	276.19	188.29	Missing	Missing	196.19	Missing	743.1	236.6	Missing	75.65
$t_{1/2}$, h	Missing	Missing	2.81	2.25	Missing	Missing	3.41	Missing	4.86	1.42	Missing	3.18
CL_{ss}/F, L/h	14.93	24.28	16.09	22.65	38.81	13.99	23.21	36.62	7.4	17.28	9.83	61.57
V_z/Fobs, L	Missing	Missing	58.7	69.06	Missing	Missing	100.4	Missing	37.73	34.68	Missing	242.51

Individual pharmacokinetic parameters for patients administered 4 g twice-daily GSR-PAS on the second occasion

Parameter	PK Parameters of GSR-PAS											
	1	2	3	4	5	6	7	8	9	10	11	12
C_{max}, µg/ml	119	55.75	55.75	52.65	40.35	65.5	36.8	33.85	69.65	30.5	27.25	12.8
t_{max},h	6	4	4	3	0	4	4	4	4	5	5	4
C_{min}, µg/ml	57.8	16.2	16.2	8.85	8.52	2.35	0.45	7.44	22.35	10.4	9.44	7.77
t_{min},h	0	0	0	0	6	0	0	1	1	8	8	2
C_{last}, µg/ml	89.3	47.5	47.5	20.25	36.95	33.65	4.84	16.85	34.75	10.4	9.44	7.99
t_{last},h	8	8	8	8	8	8	8	8	8	8	8	8
C_0, µg/ml	57.8	16.2	16.2	8.85	40.35	2.35	0.45	8.11	26.1	11.25	22.6	8.59
C_{12}, µg/ml	53.55	26.1	26.1	2	34.1	10.8	2.76	2.99	7.56	1.74	0.52	52.8
C_{24}, µg/ml	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing
AUC_{last}, µg.h/ml	726.8	295.8	297.08	276.75	181.34	305	110.08	189.4	369.13	158.93	170.04	81.01
AUC_{12}, µg.h/ml	Missing	Missing	Missing	336.51	Missing	404.5	117.44	235.99	470.12	180.86	190.09	106.82
AUC_{24}, µg.h/ml	Missing	Missing	Missing	384.88	Missing	494.94	118.91	270.03	558.91	186.66	195.43	139.15
AUC_{∞}, µg.h/ml	Missing	Missing	Missing	388.47	Missing	507.7	119.18	273	571.18	186.47	195.22	148.97
$t_{1/2}$, h	Missing	Missing	Missing	3.82	Missing	4.18	1.3	3.44	4.03	1.84	1.85	5.9
CL_{ss}/F, L/h	Missing	Missing	Missing	13.51	Missing	11.89	35.27	19.53	9.97	23.83	22.45	45.12
V_z/F_{obs}, L	Missing	Missing	Missing	56.81	Missing	47.46	63.16	72.7	40.72	56.82	54.66	228.38

Individual pharmacokinetic parameters for patients administered 4 g twice-daily GSR-PAS on the second occasion

Parameter	PK Parameters of GSR-PAS											
	13	14	15	16	17	18	19	20	21	22	23	24
C_{max}, µg/ml	41.5	71	38.2	74.1	39.85	Missing	Missing	80.25	21.45	80.5	56.3	40.1
t_{max},h	8	5	3	3	5	Missing	Missing	5	6	4	8	4
C_{min}, µg/ml	11.45	9.93	16.4	23.75	12.95	Missing	Missing	21.35	0.37	46.3	0.54	0.54
t_{min},h	5	0	2	0	0	Missing	Missing	2	2	8	0	0
C_{last}, µg/ml	41.5	38.75	25.7	49.4	30.95	Missing	Missing	61.05	20.5	46.3	56.3	17.4
t_{last},h	8	8	8	8	8	Missing	Missing	8	8	8	8	8
C_0, µg/ml	30.75	9.93	18.7	23.75	12.95	Missing	Missing	25.35	1.78	48.9	0.54	0.54
C_{12}, µg/ml	33.55	32.3	5.29	79.95	8.97	Missing	Missing	18.05	51.2	12.25	68.1	61.85
C_{24}, µg/ml	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing	Missing
AUC_{last}, µg.h/ml	161.33	328.91	228.9	459.3	221	Missing	Missing	426.33	71.06	498.5	140.55	164.8
AUC_{12}, µg.h/ml	Missing	435.47	311.11	617.07	325.93	Missing	Missing	630.76	Missing	645.83	Missing	212.92
AUC_{24}, µg.h/ml	Missing	516.33	416.16	818.68	488.21	Missing	Missing	935.08	Missing	811.49	Missing	246.24
AUC_{∞}, µg.h/ml	Missing	525.43	451.4	886.71	573.85	Missing	Missing	1081.3	Missing	847.71	Missing	248.28
$t_{1/2}$, h	Missing	3.52	6	6	7.9	Missing	Missing	7.44	Missing	5.23	Missing	3.33
CL_{ss}/F, L/h	Missing	10.99	15.8	7.91	15.95	Missing	Missing	8.25	Missing	7.37	Missing	22.12
V_z/F_{obs}, L	Missing	38.61	76.72	39.03	79.47	Missing	Missing	39.69	Missing	35.59	Missing	77.29

Individual pharmacokinetic parameters for patients administered 4 g twice-daily GSR-PAS on the second occasion

Parameter	PK Parameters of GSR-PAS				
	25	26	27	28	29
C_{max}, µg/ml	54.7	36.55	50.3	25.85	11.25
t_{max},h	3	8	4.03	5	0
C_{min}, µg/ml	2.89	0.21	14.75	0.5	1.03
t_{min},h	0	0	8	2	8
C_{last}, µg/ml	30.65	36.55	14.75	6.76	1.03
t_{last},h	8	8	8	8.05	8
C_0, µg/ml	2.89	0.21	15.6	0.76	11.25
C_{12}, µg/ml	59.35	19.61	1.18	0.45	6.24
C_{24}, µg/ml	Missing	Missing	Missing	Missing	Missing
AUC_{last}, µg.h/ml	284.64	167.21	287.94	92.73	42.09
AUC_{12}, µg.h/ml	378.02	Missing	322.46	105.35	44.64
AUC_{24}, µg.h/ml	476.4	Missing	335.6	107.82	45.58
AUC_{∞}, µg.h/ml	497.28	Missing	335.44	107.78	45.5
$t_{1/2}$, h	4.81	Missing	2.23	1.54	2.3
CL_{ss}/F, L/h	12.77	Missing	13.29	40.81	92.91
V_z/F_{obs}, L	55.81	Missing	38.4	82.63	291.16

Summary pharmacokinetic parameters for patients administered 4 g twice-daily GSR-PAS on the second occasion

Parameter	PK Parameters of GSR-PAS							
	N	Mean	SD	Min	Median	Max	CV%	Geometric Mean
C_{max}, µg/ml	39	45.47	22.13	11.25	40.3	119	49	40.22
t_{max},h	39	4.62	2.14	0	4	8	46	Missing
C_{min}, µg/ml	39	10.44	12.25	0.21	7.77	57.8	117	4.57
t_{min},h	39	2.87	3.61	0	2	12	126	Missing
C_{last}, µg/ml	39	25.7	19.86	1.03	20.5	89.3	77	17.11
t_{last},h	39	9.23	1.87	8	8	12	20	9.06
C_0, µg/ml	39	14.64	13.76	0.21	11.25	57.8	94	7.06
C_{12}, µg/ml	39	21.65	21.92	0.45	12.25	79.95	101	10.74
C_{24}, µg/ml	0	Missing	Missing	Missing	Missing	Missing	Missing	Missing
AUC_{last}, µg.h/ml	39	243.81	145.57	42.09	221	726.8	60	204.66
AUC_{12}, µg.h/ml	31	285.29	169.26	44.64	248.55	645.83	59	234.88
AUC_{24}, µg.h/ml	25	368.76	249.11	45.58	274.61	935.08	68	286.46
AUC_{∞}, µg.h/ml	25	388.28	276.3	45.5	276.19	1081.3	71	296.01
$t_{1/2}$, h	25	3.78	1.85	1.3	3.44	7.9	49	3.35
CL_{ss}/F, L/h	31	23.11	18.21	7.37	16.09	92.91	79	18.63
V_z/F_{obs}, L	25	80.73	68.26	34.68	56.82	291.16	85	65.33

Appendix K: Individual pharmacokinetic parameters of Study 2

Individual pharmacokinetic parameters for patients administered 4 g twice-daily GSR-PAS

Parameter	PK Parameters of GSR-PAS											
	MM01	CT03	EVW04	HVW05	JJ06	MB07	ES08	GS09	SB10	FF12	FA13	AS14
C_{max}, µg/ml	62	38.6	27.6	47.1	43.5	48.2	31	66.6	65.8	65.4	78.5	52.4
t_{max},h	6	0	4	3	11.98	4	8	8	3	8.03	7.98	1.98
C_{min}, µg/ml	28.5	23.1	6.66	2.02	25.4	3.87	21.4	27.8	21.6	36.5	45.5	3.79
t_{min},h	1	5.97	11.95	11.97	6	11.95	11.97	0	11.98	0	3.98	0
C_{last}, µg/ml	26.6	27.9	6.66	2.02	43.5	3.87	21.4	36.4	21.6	39.9	62.8	12
t_{last},h	12.02	11.97	11.95	11.97	11.98	11.95	11.97	11.97	11.98	11.98	11.98	12
C_0, µg/ml	35.6	38.6	12.1	11.6	42.2	12.7	23.9	27.8	24.3	36.5	72.6	3.79
C_{12}, µg/ml	26.6	27.9	6.66	2.02	43.5	3.87	21.4	36.4	21.6	39.9	62.8	12
C_{24}, µg/ml	41.1	71.6	11.7	17.2	31.6	17.7	20	40.4	36.5	44.3	50.6	12.1
AUC_{last}, µg.h/ml	557.36	340.49	197.41	304.83	413.74	293.2	315.94	617.08	503.09	586.15	799.94	383.35
AUC_{12}, µg.h/ml	556.91	Missing	197.73	304.91	Missing	293.4	Missing	Missing	503.45	Missing	Missing	383.35
AUC_{24}, µg.h/ml	710.77	Missing	229.7	308.89	Missing	303.99	Missing	Missing	626.47	Missing	Missing	438.93
AUC_{∞}, µg.h/ml	743.91	Missing	234.45	308.8	Missing	304	Missing	Missing	651.93	Missing	Missing	444.17
$t_{1/2}$, h	4.86	Missing	3.85	1.36	Missing	1.94	Missing	Missing	4.78	Missing	Missing	3.51
CL_{ss}/F, L/h	7.18	Missing	20.23	13.12	Missing	13.63	Missing	Missing	7.95	Missing	Missing	10.43
V_z/F_{obs}, L	37.71	Missing	94.88	25.42	Missing	36.74	Missing	Missing	42.28	Missing	Missing	45.64

Individual pharmacokinetic parameters for patients administered 4 g twice-daily GSR-PAS

Parameter	PK Parameters of GSR-PAS											
	WJ15	FS16	VA17	NT18	JJ19	MC20	EK22	BB24	LD25	ST26	FF27	ZL28
C_{max}, µg/ml	61.6	112	84.7	68.2	93.2	60.8	60.7	72.9	93.4	20.4	37.6	9.92
t_{max},h	4.03	1.97	3	4	6	6.08	0	8	8	12	3	11.95
C_{min}, µg/ml	35.7	51.2	6.5	12.7	31.3	8.45	1.73	39.8	21.3	3.22	13.2	0.8
t_{min},h	0	11.95	11.97	11.97	1	11.98	11.97	2.1	0	7.97	11.97	4.03
C_{last}, µg/ml	22.3	51.2	6.5	12.7	48.8	8.45	1.73	47	25.4	20.4	13.2	9.92
t_{last},h	12.07	11.95	11.97	11.97	11.98	11.98	11.97	12	11.97	12	11.97	11.95
C_0, µg/ml	35.7	100	22	28.4	37.7	14.9	60.7	49.3	21.3	17.3	33.2	1.46
C_{12}, µg/ml	22.3	51.2	6.5	12.7	48.8	8.45	1.73	47	25.4	20.4	13.2	9.92
C_{24}, µg/ml	50.4	81.9	16.2	26.6	32.9	16.5	2.36	40.6	22.4	7.38	11.2	37.2
AUC_{last}, µg.h/ml	539.32	931.53	554.42	496.23	810.7	428.12	353.23	657.33	572.53	119.3	324.31	49.77
AUC_{12}, µg.h/ml	537.82	934.09	554.64	496.66	811.53	428.27	353.28	657.33	Missing	119.3	324.75	Missing
AUC_{24}, µg.h/ml	660.84	1370.86	572.44	541.35	1134.89	452.48	356.75	Missing	Missing	Missing	398.32	Missing
AUC_{∞}, µg.h/ml	684.17	1784.04	572.53	542.54	1241.08	452.49	356.74	Missing	Missing	Missing	414.12	Missing
$t_{1/2}$, h	4.5	11.54	1.93	2.53	6.11	2	1.41	Missing	Missing	Missing	4.72	Missing
CL_{ss}/F, L/h	7.44	4.28	7.21	8.05	4.93	9.34	11.32	6.09	Missing	33.53	12.32	Missing
V_z/F_{obs}, L	37.98	37.33	19.47	26.89	28.42	25.49	22.77	Missing	Missing	Missing	65.71	Missing

Individual pharmacokinetic parameters for patients administered 4 g twice-daily GSR-PAS

Parameter	PK Parameters of GSR-PAS						
	WM29	JB30	LM31	FP33	AB34	MS02	EB23
C_{max}, µg/ml	52.9	39.7	54.4	33.4	105	Missing	62.5
t_{max},h	6	0	3.12	6	0	Missing	2.03
C_{min}, µg/ml	15.4	18.9	10.7	5.07	63.2	Missing	8.41
t_{min},h	11.98	6	12	0	11.97	Missing	12
C_{last}, µg/ml	15.4	28.6	10.7	8.75	63.2	Missing	8.41
t_{last},h	11.98	11.97	12	11.98	11.97	Missing	12
C_0, µg/ml	19.8	39.7	15.9	5.07	105	Missing	13.7
C_{12}, µg/ml	15.4	28.6	10.7	8.75	63.2	Missing	8.41
C_{24}, µg/ml	13.7	18.6	16.1	5.76	67	Missing	22.6
AUC_{last}, µg.h/ml	459.46	326.85	398.14	220.18	1020.35	Missing	415.12
AUC_{12}, µg.h/ml	459.72	Missing	398.14	220.33	Missing	Missing	415.12
AUC_{24}, µg.h/ml	526.85	Missing	458.46	256.34	Missing	Missing	458.13
AUC_{∞}, µg.h/ml	531.32	Missing	461.92	258.55	Missing	Missing	459.27
$t_{1/2}$, h	3.23	Missing	4.13	3.04	Missing	Missing	3.64
CL_{ss}/F, L/h	8.7	Missing	10.05	18.15	Missing	Missing	9.64
V_z/F_{obs}, L	35.13	Missing	51.62	67.84	Missing	Missing	45.73

Summary pharmacokinetic parameters for patients administered 4 g twice-daily GSR-PAS

Parameter	PK Parameters of GSR-PAS							
	N	Mean	SD	Min	Median	Max	CV %	Geometric Mean
C_{max}, µg/ml	30	58.33	24.16	9.92	60.75	112	41	52.59
t_{max},h	30	5.04	3.5	0	4.02	12	70	Missing
C_{min}, µg/ml	30	19.79	16.18	0.8	17.15	63.2	82	12.66
t_{min},h	30	7.25	5.19	0	9.96	12	72	Missing
C_{last}, µg/ml	30	23.58	17.93	1.73	20.9	63.2	76	16.6
t_{last},h	30	11.98	0.02	11.95	11.98	12.07	0	11.98
C_0, µg/ml	30	32.09	25.1	1.46	26.05	105	78	23.28
C_{12}, µg/ml	30	23.58	17.93	1.73	20.9	63.2	76	16.6
C_{24}, µg/ml	30	29.47	20.04	2.36	22.5	81.9	68	22.88
AUC_{last}, µg.h/ml	30	466.32	223.91	49.77	421.62	1020.35	48	404.99
AUC_{12}, µg.h/ml	20	447.54	198.46	119.3	421.69	934.09	44	404.53
AUC_{24}, µg.h/ml	18	544.8	293.76	229.7	458.29	1370.86	54	487.85
AUC_{∞}, µg.h/ml	18	580.34	379.36	234.45	460.6	1784.04	65	503.64
$t_{1/2}$, h	18	3.84	2.34	1.36	3.58	11.54	61	3.33
CL_{ss}/F, L/h	20	11.18	6.62	4.28	9.49	33.53	59	9.89
V_z/F_{obs}, L	18	41.5	18.96	19.47	37.52	94.88	46	38.14

Individual pharmacokinetic parameters for patients administered 8 g once-daily GSR-PAS

Parameter	PK Parameters of GSR-PAS											
	MM01	CT03	EVW04	HVW05	JJ06	MB07	ES08	GS09	SB10	FF12	FA13	AS14
C_{max}, µg/ml	80	118	73	66.6	67.7	61.7	65	108	91.8	125	20.7	113
t_{max},h	3	12.05	4	12	4	4	8	8	8	8	12	3.07
C_{min}, µg/ml	0.19	6.58	1.58	0	0.94	0.18	0.27	0.44	1.17	0.07	5.64	1.24
t_{min},h	0	0	0	0	0	0	0	23.98	0	24	6	0
C_{last}, µg/ml	0.55	10.7	2.63	0.03	1.31	0.42	1.23	0.44	1.38	0.07	41.7	2.61
t_{last},h	24	24.75	24	24	23.98	24	24	23.98	24	24	24.17	24
C_0, µg/ml	0.19	6.58	1.58	0	0.94	0.18	0.27	1.51	1.17	0.23	20.1	1.24
C_{12}, µg/ml	23.6	118	30.2	66.6	38.5	20.6	63.4	60.8	77.1	68.6	20.7	40
C_{24}, µg/ml	0.55	10.7	2.63	0.03	1.31	0.42	1.23	0.44	1.38	0.07	41.7	2.61
AUC_{last}, µg.h/ml	799.81	1769.21	715.13	769.25	814.95	578.65	824.91	1299.29	1216.12	1307.71	540.69	1215.79
AUC_{12}, µg.h/ml	654.94	946.07	518.15	369.48	576.42	452.73	437.13	932.36	745.24	895.66	161.09	960.75
AUC_{24}, µg.h/ml	799.81	1758.81	715.13	769.25	814.97	578.65	824.91	1299.3	1216.12	1307.71	533.76	1215.79
AUC_{∞}, µg.h/ml	801.62	Missing	729.18	Missing	820.27	580.14	829.53	1300.52	1221.06	1307.86	Missing	1227.68
$t_{1/2}$, h	2.29	Missing	3.7	Missing	2.82	2.49	2.6	1.93	2.48	1.42	Missing	3.16
CL_{ss}/F, L/h	10	4.55	11.19	10.4	9.82	13.83	9.7	6.16	6.58	6.12	14.99	6.58
V_z/F_{obs}, L	32.96	Missing	58.59	Missing	39.63	49.44	36.18	17.11	23.47	12.51	Missing	29.7

Individual pharmacokinetic parameters for patients administered 8 g once-daily GSR-PAS

Parameter	PK Parameters of GSR-PAS											
	WJ15	FS16	VA17	NT18	JJ19	MC20	EK22	BB24	LD25	ST26	FF27	ZL28
C_{max}, μg/ml	114	132	105	59.3	128	135	81.8	121	70.1	62.5	73.4	47.8
t_{max},h	4	8	6	12	6	8	4	8	7.98	8.02	8	12
C_{min}, μg/ml	16.7	21.3	1.12	0.93	2.86	0.21	0.85	0.43	0.25	0.3	0.77	3.36
t_{min},h	24	0	0	0	0	24	23.97	24	24	24	0	4.07
C_{last}, μg/ml	16.7	23.7	2.53	3.44	6.27	0.21	0.85	0.43	0.25	0.3	1.44	16.9
t_{last},h	24	24	24	24	24	24	23.97	24	24	24	24.08	23.95
C_0, μg/ml	20.5	21.3	1.12	0.93	2.86	0.34	0.95	5.95	0.36	0.55	0.77	5.87
C_{12}, μg/ml	55.8	121	59.5	59.3	82.9	91.3	64.7	90.3	44.1	26.4	56.2	47.8
C_{24}, μg/ml	16.7	23.7	2.53	3.44	6.27	0.21	0.85	0.43	0.25	0.3	1.44	16.9
AUC_{last}, μg.h/ml	1299.27	1892.72	1275.01	684.07	1532.19	1604.4	1118.83	1574.76	875.03	412.52	904.24	686.62
AUC_{12}, μg.h/ml	864.27	1024.52	902.83	307.63	997.17	1055.37	726.65	1016.6	608.93	252.31	555.99	300.04
AUC_{24}, μg.h/ml	1299.27	1892.72	1275.01	684.07	1532.19	1604.4	1118.86	1574.76	875.03	412.52	904.1	Missing
AUC_{∞}, μg.h/ml	1462.27	2100.77	1285.75	Missing	1568.62	1604.88	1121.66	1575.9	875.7	413.4	909.82	Missing
$t_{1/2}$, h	6.77	6.08	2.94	Missing	4.03	1.61	2.32	1.85	1.87	2.02	2.69	Missing
CL_{ss}/F, L/h	6.16	4.23	6.27	11.69	5.22	4.99	7.15	5.08	9.14	19.39	8.85	Missing
V_z/F_{obs}, L	53.4	33.43	26.41	Missing	29.63	11.61	23.91	13.54	24.68	56.44	34.07	Missing

Individual pharmacokinetic parameters for patients administered 8 g once-daily GSR-PAS

Parameter	PK Parameters of GSR-PAS							
	WM29	JB30	LM31	FP33	AB34	MS02	EB23	CW21
C_{max}, μg/ml	74.5	52.7	111	84.4	76.6	125	Missing	62.7
t_{max},h	8	4	6	6	12	8.1	Missing	6
C_{min}, μg/ml	0.98	0.7	0.33	1.21	28.6	3.19	Missing	0.19
t_{min},h	0	0	0	0	0	0	Missing	0
C_{last}, μg/ml	1.28	1.89	0.28	1.36	53.6	33.2	Missing	0.89
t_{last},h	24	24	24.07	24.08	24	23.92	Missing	24
C_0, μg/ml	0.98	0.7	0.33	1.21	28.6	3.19	Missing	0.19
C_{12}, μg/ml	64	21.5	32	20.9	76.6	112	Missing	54.4
C_{24}, μg/ml	1.28	1.89	0.28	1.36	53.6	33.2	Missing	0.89
AUC_{last}, μg.h/ml	1043.63	436.05	1082.98	761.4	1357.25	1949.23	Missing	886.54
AUC_{12}, μg.h/ml	651.95	295.38	888.22	626.91	576.05	1080.78	Missing	554.81
AUC_{24}, μg.h/ml	1043.63	436.05	1082.96	761.28	1357.25	1952.03	Missing	886.54
AUC_{∞}, μg.h/ml	1048.36	447.98	1083.74	767.26	Missing	2325.85	Missing	889.67
$t_{1/2}$, h	2.56	4.38	1.85	2.99	Missing	7.86	Missing	2.45
CL_{ss}/F, L/h	7.67	18.35	7.39	10.51	5.89	4.1	Missing	9.02
V_z/F_{obs}, L	28.2	112.72	19.74	44.96	Missing	39.02	Missing	31.73

Summary pharmacokinetic parameters for patients administered 8 g once-daily GSR-PAS

Parameter	PK Parameters of GSR-PAS							
	N	Mean	SD	Min	Median	Max	CV%	Geometric Mean
C_{max}, μg/ml	31	87.33	29.31	20.7	80	135	34	81.79
t_{max},h	31	7.36	2.88	3	8	12.05	39	6.79
C_{min}, μg/ml	31	3.31	6.66	0	0.93	28.6	201	Missing
t_{min},h	31	6.52	10.55	0	0	24	162	Missing
C_{last}, μg/ml	31	7.37	13.36	0.03	1.36	53.6	181	1.66
t_{last},h	31	24.03	0.14	23.92	24	24.75	1	24.03
C_0, μg/ml	31	4.22	7.51	0	0.98	28.6	178	Missing
C_{12}, μg/ml	31	58.35	28.53	20.6	59.3	121	49	51.35
C_{24}, μg/ml	31	7.37	13.36	0.03	1.36	53.6	181	1.66
AUC_{last}, μg.h/ml	31	1071.88	419.73	412.52	1043.63	1949.23	39	990.92
AUC_{12}, μg.h/ml	31	675.37	271.1	161.09	651.95	1080.78	40	611.3
AUC_{24}, μg.h/ml	30	1084.23	420.6	412.52	1063.3	1952.03	39	1002.52
AUC_{∞}, μg.h/ml	25	1131.98	467.68	413.4	1083.74	2325.85	41	1041.33
$t_{1/2}$, h	25	3.09	1.62	1.42	2.56	7.86	53	2.79
CL_{ss}/F, L/h	30	8.7	3.91	4.1	7.53	19.39	45	7.98
V_z/F_{obs}, L	25	35.32	20.78	11.61	31.73	112.72	59	30.9

Appendix L: Individual genetic results of Study 1

Summary of the genetic variants of *NAT1* in study 1

Gender	Race	NAT1 alleles	Phenotype
Female	Coloured	1*4/1*4	Fast
Male	Coloured	1*4/1*4	Fast
Male	Coloured	1*4/1*4	Fast
Male	Coloured	1*4/1*4	Fast
Female	Coloured	1*4/1*4	Fast
Female	Coloured	1*4/1*4	Fast
Female	Coloured	1*4/1*4	Fast
Male	Black	1*4/1*4	Fast
Male	Black	1*4/1*4	Fast
Male	Coloured	1*4/1*3	Unknown
Female	Coloured	1*4/1*27	Fast
Male	Coloured	1*4/1*27	Fast
Female	Coloured	1*4/1*10	Unknown
Female	Black	1*4/1*10	Unknown
Female	Coloured	1*4/1*10	Unknown
Male	Coloured	1*4/1*10	Unknown
Male	Coloured	1*4/1*10	Unknown
Male	Coloured	1*4/1*10	Unknown
Male	Black	1*4/1*10	Unknown
Male	Black	1*4/1*10	Unknown
Male	Coloured	1*4/1*10	Unknown
Male	Black	1*4/1*10	Unknown
Female	Coloured	1*4/1*10	Unknown
Female	Coloured	1*4/1*10	Unknown
Male	Black	1*4/1*10	Unknown
Female	Coloured	1*3/1*3	Unknown
Female	Coloured	1*10/1*10	Unknown
Male	Coloured	1*10/1*10	Unknown
Male	Black	1*10/1*10	Unknown

Appendix M: Individual genetic results of Study 2

Summary of the genetic variations of *NAT1 and NAT2* in study 2

Gender	Race	NAT1 alleles	Phenotype	NAT 2 Alleles	NAT 2 Phenotype
Male	Coloured	1*4/1*4	Fast	2*4/2*4	FAST
Male	Coloured	1*4/1*4	Fast	2*4/2*4	FAST
Male	Coloured	1*4/1*4	Fast	2*4/2*4	FAST
Male	Coloured	1*4/1*4	Fast	2*5/2*5	SLOW
Male	Coloured	1*4/1*4	Fast	2*4/2*6	INTERMED.
Female	Black	1*4/1*4	Fast	2*4/2*6	INTERMED.
Female	Coloured	1*4/1*4	Fast	2*5/2*5	SLOW
Male	Coloured	1*4/1*14A	Unknown	2*4/2*5	INTERMED.
Male	Coloured	1*4/1*14A	Unknown	2*4/2*7	INTERMED.
Female	Coloured	1*4/1*10	Unknown	2*6/2*7	SLOW
Female	Coloured	1*4/1*10	Unknown	2*6/2*7	SLOW
Female	Coloured	1*4/1*10	Unknown	2*4/2*5	INTERMED.
Male	Coloured	1*4/1*10	Unknown	2*4/2*5	INTERMED.
Female	Coloured	1*4/1*10	Unknown	2*4/2*4	FAST
Female	Coloured	1*4/1*10	Unknown	2*4/2*6	INTERMED.
Female	Coloured	1*4/1*10	Unknown	2*4/2*4	FAST
Male	Coloured	1*4/1*10	Unknown	2*4/2*4	FAST
Male	Black	1*4/1*10	Unknown	2*5/2*6	SLOW
Female	Coloured	1*4/1*10	Unknown	2*4/2*5	INTERMED.
Female	Coloured	1*4/1*10	Unknown	2*4/2*14	INTERMED.
Male	Coloured	1*4/1*10	Unknown	2*6/2*6	SLOW
Male	Black	1*4/1*10	Unknown	2*4/2*6	INTERMED.
Male	Black	1*4/1*10	Unknown	2*6/2*6	SLOW
Female	Coloured	1*3/1*10	Unknown	2*5/2*14	SLOW
Male	Coloured	1*10/1*10	Unknown	2*4/2*5	INTERMED.
Female	Coloured	1*10/1*10	Unknown	2*6/2*7	SLOW
Female	Coloured	1*10/1*10	Unknown	2*4/2*4	FAST
Male	Coloured	1*10/1*10	Unknown	2*4/2*5	INTERMED.
Female	Coloured	1*10/1*10	Unknown	2*4/2*5	INTERMED.
Male	Black	1*10/1*10	Unknown	2*6/2*6	SLOW
Male	Coloured	1*10/1*10	Unknown	2*5/2*5	SLOW
Male	Coloured	1088AA	not defined	2*4/2*6	INTERMED.