Determination of the effect of blood testing intervals on bioavailability and bioequivalence assessment of Fixed-Dose Drug Combination Anti-Tuberculosis Drugs

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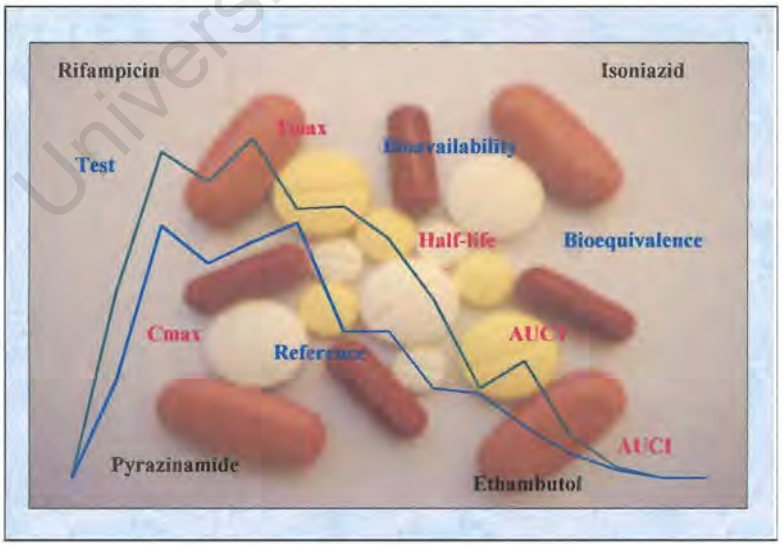
A dissertation submitted to the Department of Pharmacology, University of Cape Town in fulfilment of the requirements for the degree

Master of Science (Medicine)



Supervisors:
Professor Peter Folb
Associate Professor Peter Smith
April 2003

Dedication

In loving memory of
the late

Elizabeth February,

Arthur Ingram Mc Leod,

John Jacob van Wyk

and

Winifred Mabel van Wyk

Declaration

I, the undersigned , hereby declare that this document describes original work by the author and has not been submitted previously in any form, in its entirety or in part, to any other university for a degree. Where use was made of the work of others it has been duly acknowledged in the text.

Name and Surname: Gary Anthony Gabriels

Date: 26 June 2003

Signature: Signed by candidate

Quotations

"In the middle of difficulty lies opportunity"

Albert Einstein

"What lies behind us and what lies before us are tiny matters compared to what lies within us"

Walt Emerson

"Success comes to those who know that life is first born in thought, who seek the vision before the deed, and conforms the deed to the vision."

B.Z. Bokser

"Success, real success, in any endeavour demands more from an individual than most people are willing to offer - not more than they are capable of offering."

James Roche

"The opportunity to become who we want lies in this very moment's behaviour."

David Reynolds

"Good work that leaves the world softer, and fuller, and better than ever before, is the stuff of which human satisfaction and spiritual value are made."

Joan Chittister

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Abstract

Objective

In this study the optimum minimum time intervals and duration for testing novel fixed – dose anti-tuberculosis drugs has been determined in 13 clinical trials. Blood levels of each of the active constituents were determined in a manner that conforms with the requirements for bioavailability and bioequivalence as set out by national drug regulatory authorities. Bioavailability of isoniazid, rifampicin, pyrazinamide and ethambutol, respectively, was determined using the pharmacokinetic parameters Tmax, Cmax, half-life, AUCT and AUCI. Bioequivalence analysis was determined using the Hauschke method taking into consideration Cmax, AUCT and AUCI to determine the 90% confidence interval. The Spearman correlation coefficient verified the optimum reduced time protocol and confirmed the association of Cmax with AUCT, Cmax with AUCI and AUCT with AUCI. The entire data set allowed comparison of individual reference drugs with combined fixed drugs and made possible comparisons of the influence of sex, disease status and optimum subject number on pharmacokinetic outcomes.

Results

The findings show an optimum minimum time protocol of 6 time points; namely, 0, 1, 2, 4, 6 and 8 hours is sufficient for determining quality assurance for fixed drug combination(s) already in the market. An 11 time point protocol of 0, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6 and 8 hours provided sufficient information and is comparable to the conventional 15 time points. Comparison of the overall data set of isoniazid and rifampicin yielded significantly higher values for Cmax of the single reference principal compared with the same active principal in fixed combination. The mean Cmax values were significantly higher for rifampicin and pyrazinamide, regardless of formulation, in females compared to males. Healthy volunteers attained significantly higher mean Cmax values for rifampicin than did patients with tuberculosis. The minimum required subject number for investigation of a given fixed-dose drug combination consisting of isoniazid, rifampicin and pyrazinamide was found to be 18 subjects.

Publications, Reports and Conference Presentations

Parts of the work presented in this thesis have been published , presented as reports and at conferences as follows:

Publications

McIlleron, H., **Gabriels, G.**, Smith, P. , E., Fourie, P.B. Ellard, G. (1999) The development of a standardised screening protocol for the in vivo assessment of rifampicin bioavailability Int. J. Tuberc Lung Dis, 3(11): S329-S335.

Pillai, G., Fourie, P.B., Padayatchi, N., Onyebujoh, P.C., McIlleron, H., Smith, P., **Gabriels, G.** (1999). Recent bioequivalence studies on fixed-dose combination anti-tuberculosis drug formulations available on the global market. Int. J. Tuberc Lung Dis, 3(11): S309-S316.

Reports

Fourie, B., Weyer, K., Smith, P., Barnes, K., Blockman, M., **Gabriels, G.**, Becker, P., (1996). Bioavailability of Isoniazid and Rifampicin in two fixed dose formulations (Rifinah 150 and Rifinah 300), compared to the bioavailability of reference formulations of the two agents administered separately.

McIlleron, H., Smith, P., **Gabriels, G.**, Viljoen, E., Fourie, P.B. (1997). Bioavailability and Bioequivalence of Isoniazid, Rifampicin and Pyrazinamide in fixed dose formulation (Rifcin Co), compared to the bioavailability of single drug reference preparations of the three agents.

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McIlleron, H., Smith, P., **Gabriels, G.**, Viljoen, E., Fourie, P.B. (1997). Bioavailability and Bioequivalence of Isoniazid, Rifampicin in fixed dose formulation (Rifcin Ped), compared to the bioavailability of single drug reference preparations of the two agents.

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McIlleron, H., Lawrenson, S., Smith, P., **Gabriels, G.**, Viljoen, E., Fourie, P.B. (1998). Bioavailability study comparing Rifinah combination paediatric powder (Rifampicin 60mg and Isoniazid 30mg) as single dose to equivalent doses of Norstan-Isonizid , Rifadin as a single dose, in healthy volunteers.

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McIlleron, H., Lawrenson, S., Smith, P., **Gabriels, G.**, Viljoen, E., Fourie, P.B. (1998). Bioavailability study comparing Rifinah combination tablets (Rifampicin 150mg and Isoniazid 150mg) as a single dose to equivalent doses of Norstan-Isonizid® , Rifadin® as a single dose, in healthy volunteers.

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Conferences

H McIlleron, P Wash, J Cockcroft, J Wilkins, A Fredericks, J van Dyk, G **Gabriels**, A Burger, P Folb, P Smith - Low and variable rifampicin levels in tuberculosis patients. International Conference TB Strategies for Africa-18th-20th October 2000, Cape Town, South Africa.

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Abbreviations

WHO	World Health Organisation
MCC	Medicines Control Council
HPLC	High performance liquid chromatography
TB	Tuberculosis
INH	Isoniazid
RIF	Rifampicin
DRIF	Desacetyl rifampicin
PZA	Pyrazinamide
ETB	Ethambutol
STREP	Streptomycin
DOTS	Directly observed treatment strategy
MIC	Minimum inhibitory concentration
MBC	Minimal bactericidal concentration
SEM	Standard error of the mean
GMP	Good manufacturing practice
Cmax	Maximum concentration
Tmax	Time at which maximum concentration is reached
AUC	Area under curve
AUCT	Total area under the curve as from the time of dosing to the time of the last observation.
AUCI	Area under curve extrapolated to infinity
T1/2	half-life
FDC	Fixed-dose drug combination
REML	Residual maximum likelihood

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Chapter 1
Introduction

Tuberculosis is an ancient human disease but efforts to bring this devastating illness under control have been only partly successful. For this reason, policy, intervention, management and discovery of new treatments will remain of paramount importance.

The essential anti-tuberculosis drugs investigated in this study were isoniazid, rifampicin, pyrazinamide and ethambutol which are first-line drugs currently used for anti-tuberculosis treatment.

1.1 History of Tuberculosis

Tuberculosis is responsible for some of the greatest morbidity and mortality of all infectious diseases that plague humans today. In ancient Hindu texts, tuberculosis is referred to as Rogaraj, the king of disease, and Rajayakshma, the disease of kings. The first of these, like John Bunyan's "Captain of all of these Men of Death", emphasises that tuberculosis is still in the forefront of the disease burden. The second name stresses the fact that tuberculosis strikes indiscriminately, affecting monarch and peasant [2,4,10,63,128,131].

Tuberculosis surveillance in many countries is inadequate for determining accurate numbers. The burden of TB is estimated indirectly using several epidemiological measurements. These measurements include average annual risk of infection, reported incidence of smear-positive disease, estimated cover of population by health services, estimated proportion of all smear-positive cases, and the case fatality rates for smear-positive and other forms of TB [2, 138, 12, 171, 175].

The World Health Organisation estimates that there are as many as 20 million active cases of tuberculosis worldwide. Active cases annually infect an estimated 50-100 million people in areas of high prevalence. Approximately 3 million people die every year of tuberculosis, more than 80% of whom live in developing countries [1]. Deaths from tuberculosis account for 25% of all avoidable deaths in developing countries, and 75% of these cases occur in people who are economically productive [7, 18, 21, 163].

For many, tuberculosis is a dreaded deadly disease, reminiscent of the attitude to tuberculosis during the Industrial Revolution [10, 31, 168, 169].

Mycobacterium tuberculosis is the most important of the mycobacterial pathogens in human disease. However, a number of other mycobacteria can produce pulmonary or other disease in man indistinguishable from infection caused by *Mycobacterium tuberculosis* [1, 60].

The global magnitude of the TB problem is enormous [141]. Its economic impact reflects the direct and indirect costs of treatment of active and suspected cases, contact investigations, screening, preventative therapy programmes, hospital and institutional control, cost of lost income, loss of employment, decreased likelihood of marriage (especially for women), and creation of orphans and one-parent households [13, 32, 33, 54, 127, 170,172].

1.2 Treatment of Tuberculosis

On March 24th 1882 when Robert Koch, after describing the series of experiments that led to the discovery of the organism responsible for tuberculosis, concluded that, to control the disease, the sources from which the infectious material flows must be closed as far as possible. The most important of these sources, is the sputum of patients. This was a landmark in the chemotherapy of tuberculosis [11, 132].

Anti-tuberculosis drugs should ideally have three properties: bactericidal ability, sterilising activity and prevention of resistance [55]. The susceptibility of the mycobacterium depends on access of the drug to the site where it is needed and hence, for successful treatment, it is necessary that the formulation is adequate for delivery [134]. Useful in achieving efficient drug uptake and concentration at the site of the disease would be a pro-drug that is converted to the active form in the bacteria [2, 26, 155]. The benefit a highly effective anti-tuberculosis drug, or combination of drugs, should provide better therapy, lower cost and easy supply.

The objective of tuberculosis control policy is to reduce mortality, morbidity, prevent relapse, disease transmission and drug resistance [133]. TB control aims at achieving standardised short-course chemotherapy (SCC) under direct observation, at least during the initial phase of treatment, for all smear-positive TB cases [6].

The success of directly observed treatment strategy (DOTS), in addressing the global tuberculosis emergency, depends on a five-point approach. This includes government commitment to a national tuberculosis programme, adequate provision for case detection, effective implementation of short-course chemotherapy, uninterrupted supply of essential anti-tuberculosis drugs, and

monitoring outcome [41, 57, 130, 177].

A number of anti-tuberculosis formulations is currently available. It is important that ensuring appropriate drug dose in combined formulation patient-weight should be considered, and that the training and experience of health care personnel, the health care coverage and available resources should form part of the overall strategy of care, and of procurement of these drugs from a national list of essential drugs [7].

The consequence of famine, war and natural disasters is the creation of large populations of displaced, malnourished people in crowded living conditions, who are especially predisposed to tuberculosis [77, 131, 142]. Furthermore, where immunity of the population is undermined by HIV infection, the incidence will rise [25, 28, 34, 41, 62, 71, 126, 162, 168, 169].

1.3 Treatment with anti-tuberculosis drug combinations

Isoniazid (INH) is a potent anti-tuberculosis drug with specificity for *M tuberculosis* [66, 75, 81, 83]. It is effective in achieving sterilisation of the sputum in the early treatment phase. There is considerable literature concerning the mode of action [3]. The principal form of toxicity is hepatic injury, which can be severe and even fatal [5, 30]. Rifampicin is a potent antimycobacterial drug with a broad therapy antibacterial spectrum, and it is especially important in the continuation phase [91].

Pyrazinamide is bactericidal against certain populations of mycobacterium [67, 93, 147]. Ethambutol has a polyamine structure. This drug was originally thought to

interfere with RNA synthesis, but recent evidence from metabolic labelling suggests the mechanism to be the inhibition of glucose incorporation. Its sterilising activity is bacteriostatic [3, 43, 45, 46, 139].

Effective treatment requires three to five drugs given concurrently. These drugs may be administered as single-drug formulations or in fixed–dose combinations, where two or more drugs are in fixed proportions.

Drug combinations such INH and PZA have demonstrated empirically that they are synergistic, making it attractive to propose rational strategies for the design of potentially useful combinations. Combination therapy may be beneficial in that inhibitors disrupting an aspect of cell wall biosynthesis may not necessarily be lethal themselves, but might affect the permeability of other drugs [2, 35]. The rifamycins significantly enhance the activity of isoniazid [89,154]. For TB chemotherapy to succeed, it must not only prevent drug resistance, but also kill all organisms to prevent their subsequent multiplication and relapse [48,49, 74, 103].

In practice, the efficacy of chemotherapy is reduced by a number of factors. These include hospitalisation, lack of adequate drug supply and delivery, and diagnostic facilities. Effective tuberculosis treatment is highly successful in terms of medical science, but successful implementation also depends on socio-economic considerations that relate, in turn, to political decisions [11, 162].

In deciding on the optimum therapeutic regimen, correct amounts of each drug need to be determined and individualised [7].

While DOTS serves an important purpose, fixed-dose combination drugs may alleviate treatment difficulties [79, 143].

Treatment failure can be attributed to a number of factors, including non-compliance [50, 78]. This in drug dependence require a detailed addiction history[11, 90, 153, 164, 166, 173]. Mycobacterium develop resistance as a result of exposure to less than the minimum inhibitory concentration (MIC) of drug over a sustained period of time [47, 64, 72].

DOTS has been effective; however, where there is a shortage of suitably trained personnel to ensure that the necessary procedures are followed, DOTS has been less successful [80].

DOTS does not optimise individual treatment of tuberculosis, but instead it represents a standardised treatment regime that covers a number of variables [167].

Public health authorities have recognised the need to improve the nutritional status of tuberculosis patients, and supervised food intake, together with administration of drug has been introduced. However, there is a risk that food alters drug kinetics, decreasing bioavailability and compromising the efficacy of treatment by sub-therapeutic levels, leading to resistance [68, 73, 82]. Prolonged treatment regimens may undermine morale, resulting in non-compliance and failure to achieve effective eradication of the organism [29,167].

Where both HIV infection and *M. tuberculosis* infection are common [8, 27, 165], and where poor quality drugs or counterfeit drugs are available, a negative outcome of treatment is to be anticipated [174].

1.4 Fixed-Dose Combinations (FDCs)

The WHO together with the International Union Against Tuberculosis and Lung Disease (IUALTD) have advocated replacement of single-drug preparations by FDCs in primary treatment of tuberculosis [1-5, 105, 109, 113].

The justification for recommending that FDCs replace single drugs in primary treatment of tuberculosis includes the following:

- FDCs obviate monotherapy, reducing emergence of drug-resistant tuberculosis.
- FDCs simplify treatment, reduce prescription error and increase compliance.
- FDCs simplify drug stock management, procurement and distribution.
- FDCs reduce the risk of rifampicin for treatment of conditions other than tuberculosis.

Prevention of drug resistance

Emergence of drug resistance in high-burden areas of the world is a major threat to the future success of TB control. Drug resistance in most tuberculosis patients is predominantly the result of multiple interruptions in treatment [56]. With single drugs, patients are prone to interrupt their treatment with some drugs and not with others, creating a risk of selection of drug resistance. Expiry of supplies leads to some drugs being continued without others - another cause of monotherapy.

As drug resistance and treatment failure increase, it is imperative that there should be sound policies and procedures in place for managing existing drugs efficiently [3, 5, 70, 106, 167].

FDCs of good quality should help prevent drug resistance when given as directly observed treatment as part of the DOTS strategy. Ensuring adequate bioavailability of rifampicin is essential [111]. If other drugs in FDCs are included in the evaluation, this will improve the prospects of achieving a good quality product. Bioavailability data is essential for this purpose [110].

Simplifying treatment

Having to swallow fewer pills each day would make treatment easier (Table 1). FDCs improve the selection of drugs and may reduce mistakes in calculation of dose [178].

Table 1. Tablets taken in the intensive treatment phase by a 50kg patient

Single–drug tablets	Number of tablets	FDC tablets	Number of tablets
Rifampicin 150mg Isoniazid 300mg (100)mg Pyrazinamide 400mg Ethambutol 400mg (100)mg	3 1(3) 3 2(7)	Rifampicin, Isoniazid, Pyrazinamide, Ethambutol (150mg+ 75mg+400mg +275mg)	3
Total	9 (16)		3

* Figures in parenthesis refer to alternative dose formulations and the related number of tablets

Simplifying drug supply

FDC tablets simplify management of drug supply. With single drugs, out-of-stock situations occur for three reasons: no buffer stock, delays in receipt of orders, and expiry date reached without replacement stock being available. With FDCs, there are fewer drugs to consider, making it easier to calculate drug needs. Because there are fewer drug formulations to order, ship and distribute, there is less strain on staff. Adverse effects are not more commonly reported for FDC tablets than single-drug combinations [179, 180].

FDCs minimise the risk of misuse of rifampicin for conditions other than tuberculosis

Besides tuberculosis, several other common infectious diseases can be treated successfully with rifampicin. Theft and illegal sale of this drug are not uncommon. FDC tablets are much less attractive for sale on the black-market [177]. FDC formulations may be tablets, capsules or paediatric suspensions, and are manufactured in some countries for local use and for export.

1.5 Quality of FDC tablets

It is essential that only FDC tablets of good quality should be used [124]. Satisfactory *in vitro* dissolution testing does not necessarily assure acceptable bioavailability of the constituent drugs [69]. In a series of studies, some FDCs had acceptable rifampicin bioavailability and others did not [23, 24, 59, 69, 108, 109, 125, 149, 181, 182]. The quality of any drug depends critically on good manufacturing practice (GMP) [17, 98], without which quantities specified by the manufacturer are not necessarily acquired. Furthermore the physical characteristics of the crystalline structure of rifampicin contribute to effective concentration of the drug in the blood. The starting materials in the manufacturing process can also negatively affect bioavailability [19, 88].

The bioavailability of rifampicin is particularly at risk if strict manufacturing procedures are not followed, or poor quality raw materials are used. Poor bioavailability leads to unsatisfactory outcomes and may predispose to drug resistance. Good quality FDCs, with demonstrated bioavailability of rifampicin and other drugs, are a prerequisite for successful treatment outcomes with FDC-based regimens [113, 140].

Against this background, WHO and IUATLD issued a joint statement in 1994 advising that only FDC tablets of good quality and proven bioavailability of rifampicin should be used in the treatment of tuberculosis [156].

Pre-qualification of manufacturers is widely accepted as necessary, requiring clear specifications, high quality product testing and adequate monitoring. Once a pre–qualification scheme for anti-tuberculosis drug manufacturers has been established and implemented, procurement agencies would be able to purchase exclusively from pre–qualified manufacturers to assure quality.

Bioavailability of rifampicin should always be demonstrated for registration approval of FDC, and added benefit would be achieved if this is also done for the other anti-tuberculosis drugs confined in the FDC, using a simplified protocol. No correlates of bioavailability are known that do not involve human subjects in assessing drug kinetics. Therefore, the assessment of drug absorption and uptake can only be done through clinical studies. Although dissolution testing is useful in evaluating lot-to-lot consistency, it cannot replace bioavailability studies.

1.6 Regulatory approval of FDC Tablets

The registration process is vitally necessary to ensure that only drugs of good quality are purchased and used. Not only should the product be of good quality, but it also should adhere to GMP and quality control [17]. WHO and partners are working to strengthen prompt registration of FDC [84-87, 122]. Other measures include maintenance of a laboratory network for quality assurance of FDC tablets [118, 121]. It is increasingly required that bioavailability testing should be performed for all components of the FDC tablet.

The WHO certificate of quality of pharmaceutical product(s) should be attached to imported products. The necessary specifications should be presented and good storage practices adhered to. If regulations are weak and enforcement poor, the potential for counterfeit and substandard drugs is real.

1.7 The market for FDC tablets

Since Acocella presented his work in Dubrovnik [113,177] FDCs are increasingly used for the treatment of TB. A WHO survey of the global market for FDCs in 1998 estimated that approximately 50% of countries used FDCs [106, 107]. According to a 1998 WHO survey, an estimated 24% of TB cases worldwide were treated with rifampicin-containing FDC tablets. However, most patients receive the two–drug combination. Less than 5% of cases are given three- or four-drug FDCs with 75% of cases still being treated with single drugs. It is a major task to replace single tablets with FDCs in the treatment of TB.

1.8 Currents concerns with FDCs and the need for bioavailability and bioequivalence testing

The WHO has repeatedly emphasized that, to confront the global tuberculosis emergency, all the elements of the framework of tuberculosis control should function optimally [136]. An essential component of the DOTS strategy is to have a reliable supply of good quality drugs. Using FDCs with poor bioavailability leads to poor treatment outcomes and drug resistance. The benefits of improved treatment, reduced logistical cost and the prevention of MDR-TB further justify the change to FDCs.

The need to establish adequate bioavailability of rifampicin in FDCs has long been recognised. Concern has been expressed about apparent inadequate bioavailability of rifampicin in some FDCs preparations [6-8, 14-16, 109]. Consequently, international regulatory authorities recommend that the bioavailability of formulations be compared with that of preparations of reputable efficacy. Only those that have shown to be bioequivalent should be released onto the market [7, 9].

Whilst over the years focus has been placed on the bioavailability of rifampicin within FDCs it is very often assumed that other anti-tuberculosis drugs INH, PZA and ETB perform acceptably within the FDC as part of ongoing quality assurance procedures. Efficient and standardised methods should test the quality of all drugs that constitute the FDC for regulatory approval and for ongoing quality assurance procedures [129].

Minimum time-point schedule protocol for conducting bioequivalence studies of rifampicin using limited sampling time points, showed limited loss of precision [113, 114].

The pharmacokinetic comparative, relative to an existing time schedule protocol aimed at reducing the number of sampling time points, not just for rifampicin, needs to be efficient to evaluate formulations currently in use in tuberculosis programmes as well as those needing approval by the regulator [8, 109].

Linked to effective policy, it is essential that low cost quality assurance and control procedures form part of standard operating procedures. This is important in both medical and commercial terms as it would assist the process of adequate delivery as well as the correct quantity of anti-tuberculosis drugs to patients [120, 135].

1.9 Factors contributing to the need for minimum time-point quality assurance procedures

(i) At present it is easy for larger pharmaceutical industries to manufacture FDCs used in an extended time point protocol, but for smaller industries cost becomes a factor.

(ii) This research addresses the need to determine the minimum number of time points for quality assurance procedures in the investigation of FDC bioavailability, using validated assay methods according to accepted standardized protocols.

A validated bioavailability and bioequivalence protocol that uses minimum time points would reduce development time and cost, simplify regulatory requirements, achieve standardization, and ensure bioavailability of rifampicin and other anti-tuberculosis drug components. To demonstrate consistency in quality, the principal parameters of interest should be peak serum levels (Cmax) and area under the curve (AUC).

The system should be simple, low in cost, and rapid to ensure that pre-qualification criteria do not act as a deterrent to manufacturers producing anti-TB FDC. Whilst manufacturers support a system of strict quality assurance on rifampicin-containing FDC, if other anti-tuberculosis drugs within the given FDC can be determined in a simplified way, then this will certainly give added confidence to the formulation.

With the monitoring of FDCs in the market place by independent laboratories, bioavailability and bioequivalence testing would meet the required standards on a continual basis [7, 9, 115-117, 123, 159]. A simplified minimum time-point protocol would facilitate such a policy.

Objective and Aims

Objective

To investigate a number of screening-time protocols of anti-tuberculosis fixed-dose drug combination formulations to determine the minimum number of time points required to confirm bioavailability and bioequivalence relative to an existing 15 time point protocol.

Aims

(i) To compare the bioavailability of 13 fixed-dose drug formulations using an existing protocol, approved by the national regulatory authority, to that of known reference formulations. The following parameters would be compared, using concentration-time curves: Tmax, Cmax, half-life, AUCT and AUCI.

(ii) To use the information obtained to assess which of the fixed-dose drug formulations show bioequivalence to reference combinations.

(iii) To establish the minimum number of time points, in a protocol containing fewer time points than conventional and to compare the bioavailability and bioequivalence data obtained with that of longer duration protocols.

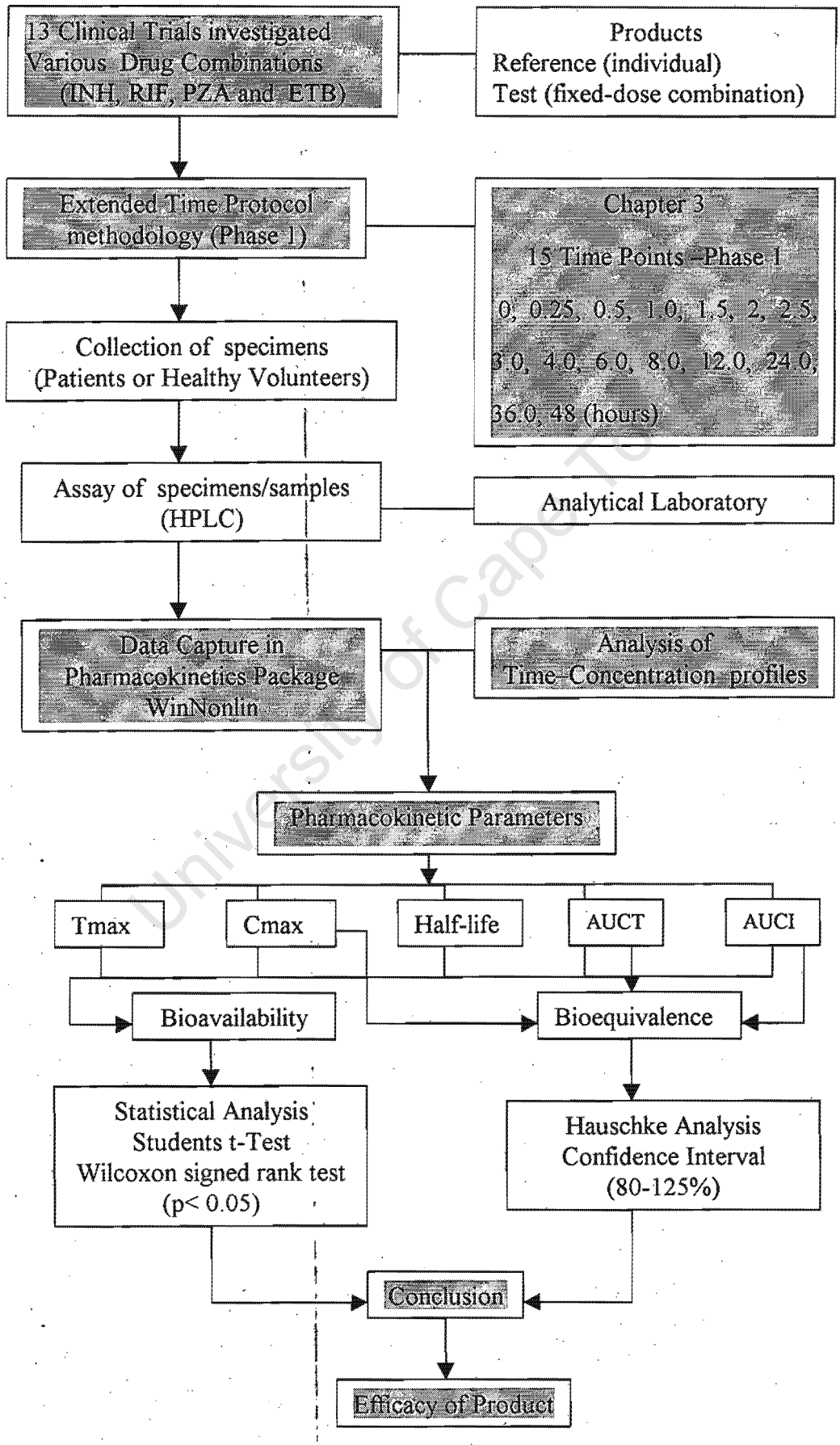
(iv) To do comparative correlation analysis on Cmax, AUCT and AUCI, to determine the minimum number of time points.

(v) To conduct subgroup analysis by investigating the number of fixed-dose drug formulations as an inclusive data set, for comparison with a number of demographic variables.

(vi) To conduct subject reduction analysis to determine the minimum number of subjects necessary for bioavailability and bioequivalence testing of fixed-dose drug formulations.

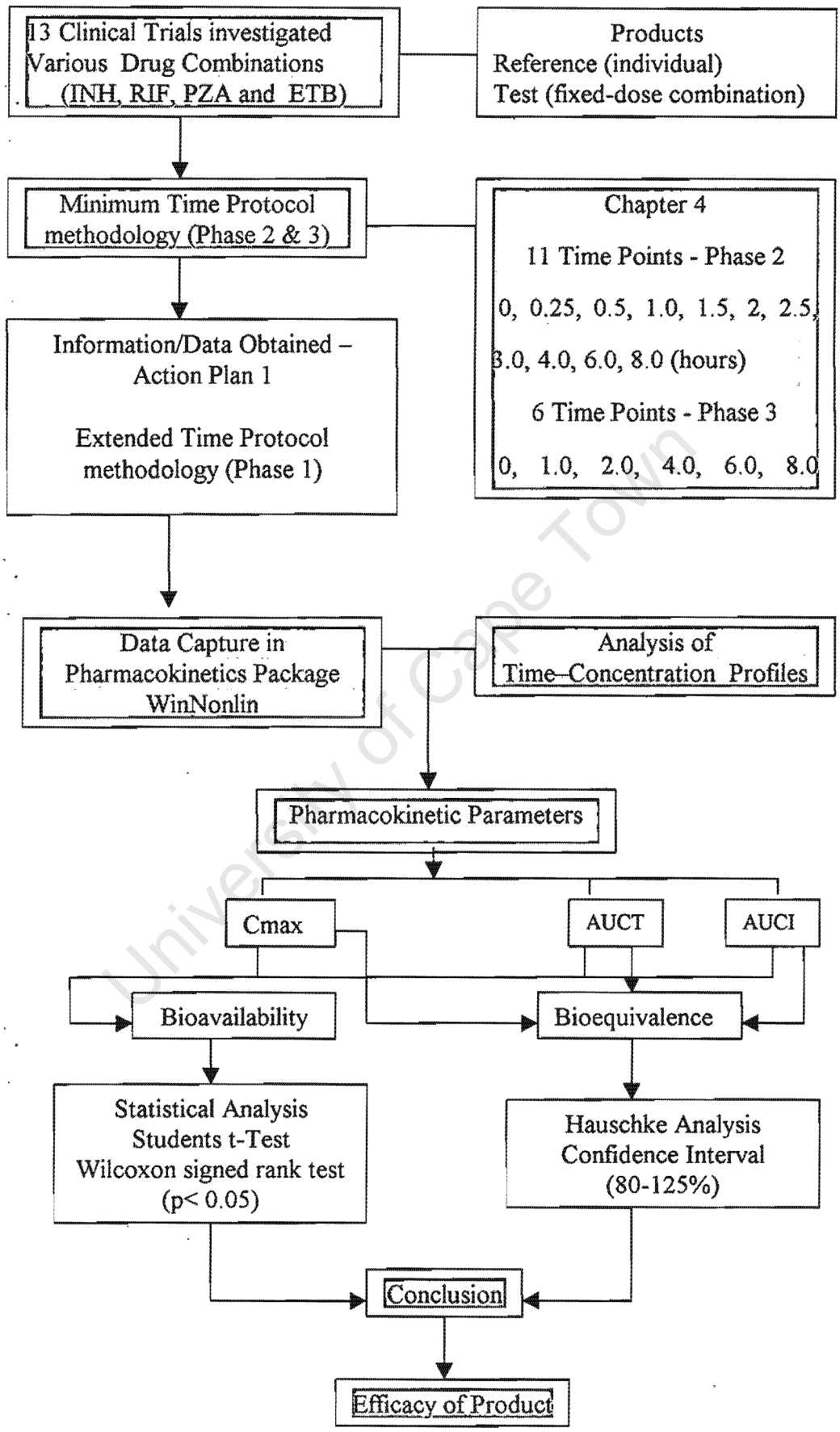
Action Plan 1

Objective: To accomplish aim (i) and (ii) of Objective and Aims



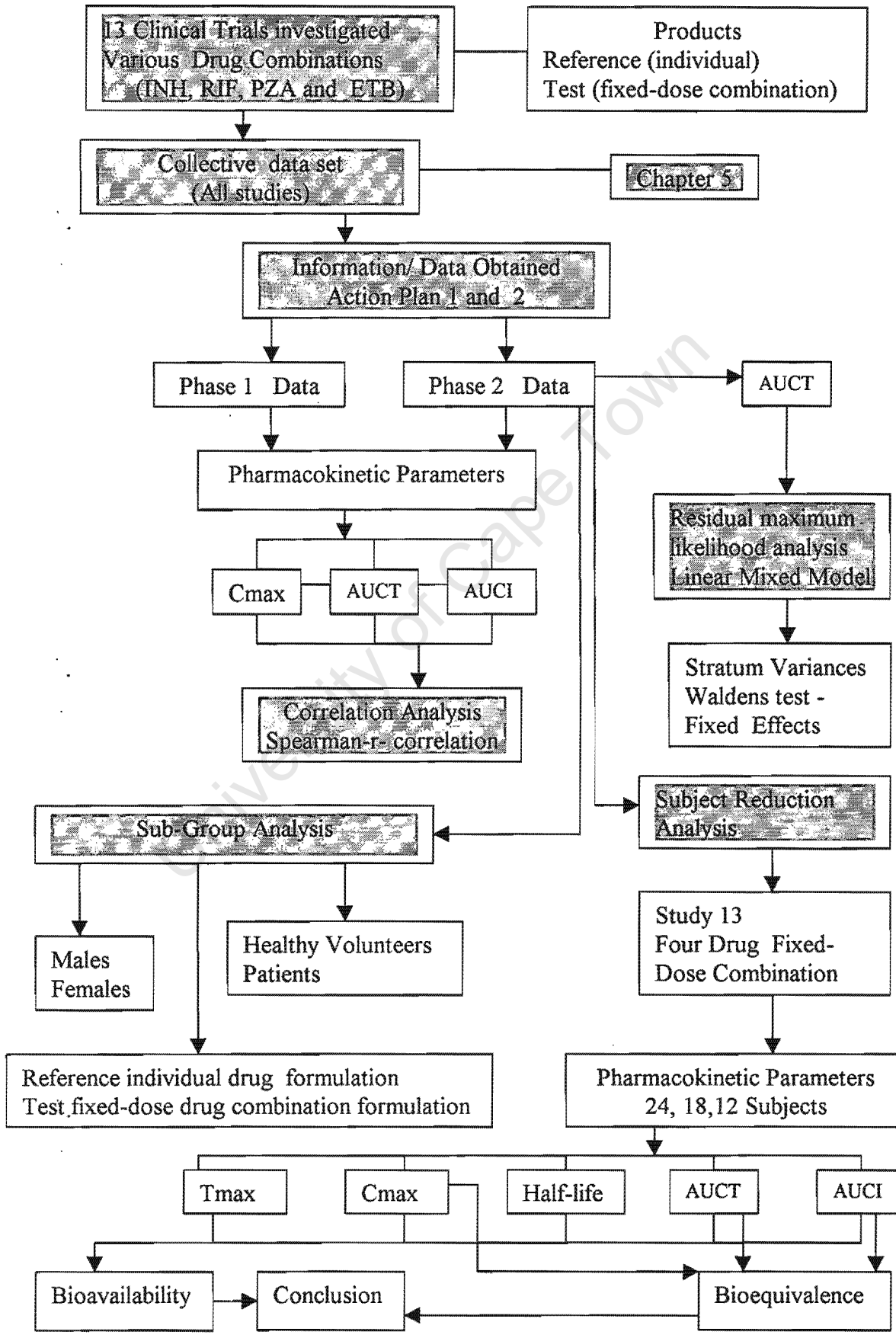
Action Plan 2

Objective: To accomplish aim (iii) of Objective and Aims



Action Plan 3

Objective: To accomplish aim (iv) and (v) of Objective and Aims



Chapter 2
Methodology

The study used commercial computerised application software packages as a tool to determine pharmacokinetic parameters in a comparative way, that allowed for the determination of the optimum minimum time protocol. The parameters obtained allowed for the investigation of bioavailability and bioequivalence results of fixed-dose drug combinations and how this compared to the necessary required standards.

2.1 Studies Investigated

Table 2 indicates the reference and test products, together with the source, that were utilised in each of the studies, the dosages that constituted the fixed-dose drug combinations and the number of anti-tuberculosis drugs in each. Appendix 4, presents the randomisation sequence that was used for the first period of each of the respective studies [99]. The reverse sequence was used for the second period after the washout period. The exception was Study 1 which was not a classical double cross over design , but instead extended across three periods.

Table 2. FDC, dose and reference product source for studies investigated

	Test Product	Test Product Dose (mg)	Reference Product	Dose (mg)	Total Dose (mg)
Study 1 Rifinah 150 July 1996 Healthy Volunteers (16) Gruppo Lepetit	INH RIF	4x100 4x150	Isoniazid (Lennon) Rifacap (Quatromed)	4x100 4x150	400 600

	Test Product	Test Product Dose (mg)	Reference Product	Reference Product Dose (mg)	Total Dose (mg)
Study 1 Rifinah 300 July 1996 Healthy Volunteers (16) Gruppo Lepetit	INH RIF	2x150 2x300	Isoniazid (Lennon) Rifacap (Quatromed)	3x100 4x150	300 600
Study 2 Myrin-P December 1996 Healthy Volunteers (24) Lederle	INH RIF PZA ETH	5x60 5x120 5x300 5x225	Isoniazid (Lennon) Rimactane (Ciba) Rozide (Rolab) Mycrol (Rolab) Mycrol (Rolab)	3x100 4x150 3x500 2x400 3.25x100	300 600 1500 1125
Study 3 Rifcin Pedz February 1997 TB Patients (16) Ciba	INH RIF PZA	10x30 10x60 10x150	Isoniazid (Lennon) Rimactane (Ciba) Rozide (Rolab)	3x100 4x150 3x500	300 600 1500
Study 4 Rifcin Co April 1997 TB Patients (22) Ciba	INH RIF PZA	5x60 5x120 5x300	Isoniazid (Lennon) Rimactane (Ciba) Rozide (Rolab)	3x100 4x150 3x500	300 600 1500
Study 5 Rifcin Ped April 1997 TB Patients (19) Ciba	INH RIF	10X60 10x60	Isoniazid (Lennon) Rimactane (Ciba)	6x100 4x150	600 600

	Test Product	Test Product Dose (mg)	Reference Product	Reference Product Dose (mg)	Total Dose (mg)
Study 6 Rifadin July 1997 Healthy Volunteers (24) HMR	RIF	1x600	Rimactane (Ciba)	1x600	600
Study 7 Rifafour October 1997 Healthy Volunteers (23) HMR	INH RIF PZA ETH	5x60 5x120 5x300 5x200	Norstan-Isoniazid (Ciba) Rimactane (Ciba) Rozide (Rolab) Mycrol (Rolab) Mycrol (Rolab)	3x100 1x600 3x500 2x400 2x100	300 600 1500 1000
Study 8 WHO April 1998 Healthy Volunteers (19) WHO	INH RIF PZA	5x50 5x120 5x300	Isoniazid (Evans Medical) Isoniazid (Evans Medical) Rimactane (Ciba) Rozide (Rolab)	2x100 1x50 4x150 3x500	 250 600 1500
Study 9 Rifinah Ped June 1998 Healthy Volunteers (24) HMR	INH RIF	10x30 10x60	Norstan-Isoniazid (HMR) Rifadin (HMR)	3x100 1x600	300 600

	Test Product	Test Product Dose (mg)	Reference Product	Reference Product Dose (mg)	Total Dose (mg)
Study 10 Rifater Ped August 1998 Healthy Volunteers (21) HMR	INH RIF PZA	10x30 10x60 10x150	Norstan-Isoniazid (HMR) Rifadin (HMR) Rozide (Rolab)	3x100 1x600 3x500	300 600 1500
Study 11 Rifinah150/150 November 1998 Healthy Volunteers (22) HMR	INH RIF	4x150 4x150	Norstan-Isoniazid (HMR) Rifadin (HMR)	6x100 1x600	600 600
Study 12 Rifinah150/75 October 1998 Healthy Volunteers (22) HMR	INH RIF	4x75 4x150	Norstan-Isoniazid (HMR) Rifadin (HMR)	3x100 1x600	300 600
Study 13 Rifafour E275 January 1999 Healthy Volunteers (24) HMR	INH RIF PZA ETH	4x75 4x150 4x400 4x275	Norstan-Isoniazid (HMR) Rifadin (HMR) Pyrazinamide (HMR) Rolab-Ethambutol (Rolab) Rolab-Ethambutol (Rolab)	3x100 1x600 4x400 2x400 3x100	300 600 1600 1100

2.2 Clinical Methodology

The clinical trials conducted in the Department of Pharmacology, University of Cape Town since 1996 which followed internationally accepted standardised principles for bioavailability and bioequivalence testing [16, 17, 19, 144, 145] and monitoring [25,26, 28] were investigated or validated as part of ongoing research 95-97, 101, 157, 160]. Ethical approval from the Research Ethics Committee of the University of Cape Town and the Medicines Control Council had been obtained prior to the start of each study.

The studies included 2, 3 and 4 fixed-dose drug combinations consisting of rifampicin, isoniazid, pyrazinamide and ethambutol, respectively, given as a single reference principal and as fixed combination active principal one week apart to the same subjects in a randomised open within-subject cross over design [102, 150, 151]. 10 studies were investigated in healthy volunteers and 3 in patients, all of whom had fulfilled the inclusion, and none of the exclusion criteria and had given written informed consent (Appendix 1).

It is important to have an understanding of the exact function and purpose of the individual drug constituents, in the formulation of fixed-dose anti-tuberculosis combinations [76]. Table 3 modified from Davidson and Quoc Le's review article, grades anti-tuberculosis drugs into different classes and gives an overview[40, 61].

Table 3. Classes and Grading of anti-tuberculosis drugs

Class of anti-tuberculosis drugs			
	1	2	3
Description of Classes	Drugs with resistance prevention	Drugs with early bactericidal activity	Drugs with sterilising activity
Drug and rank in decreased order of effectiveness	INH RIF ETB	INH ETB RIF	RIF PZA INH ETB
Comment	These agents when combined can prevent the emergence of resistant mutants to the companion drug.	These drugs induce rapid decrease in the number of living bacilli in the sputum at the beginning of treatment to sputum negative cultures thus reducing risk of transmission.	Drugs having the ability to kill all tubercle bacilli in the lesions of experimental animals (probably humans) reducing the relapse rate to a minimum within a short period.

2.3 Analytical Methodology

Specimen collection of venous blood was obtained at the sampling times that were required for the clinical study and the sensitive high performance liquid chromatography (HPLC) method of Zent and Smith [29] was used by the analytical laboratory in the Department of Pharmacology, University of Cape Town to analyse for rifampicin, isoniazid and pyrazinamide. For ethambutol the modified method of Lee and Wang [22, 65, 104, 112, 158] was used. Figure 1 depicts the chemical structure of the anti-tuberculosis drugs investigated. Isoniazid

and pyrazinamide are similar in chemical structure and size, making them attractive synergistic drugs in the design of combinations. Ethambutol has a more polyamine linear chemical structure, while rifampicin has a larger molecular structure compared to the other drugs investigated and is potentially vulnerable in crystalline form due to polymorphism [88].

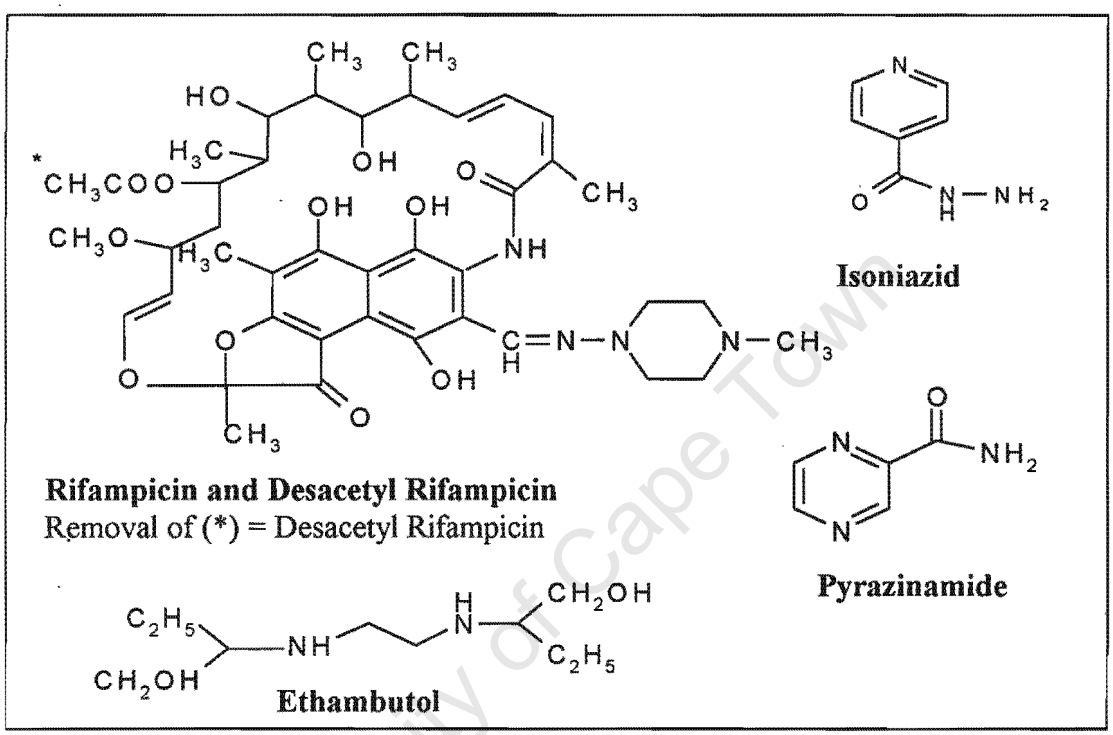


Fig 1. Chemical structure of anti-tuberculosis drugs investigated

2.4 Statistical and Pharmacokinetic Analysis Methodology

Upon completion of the analytical and assay methodology, using HPLC analysis, data obtained for the 13 respective clinical trials was captured or validated by the investigator using a computer-based widows program WinNonlin Standard Edition Version 1.5 (WinNonlin © 1984-1997, Scientific Consulting, Inc), developed to evaluate data using nonlinear modelling.

The model uses one or more partial derivatives to determine parameters from compartments that either have linear or non-linear kinetics [161]. This makes the application suitable for pharmacokinetic and/or pharmacodynamic modelling and noncompartmental analysis [94, 152].

Taking into consideration that a single dose was administered at time zero, noncompartmental or model independent analysis described the concentration-time curve by estimating noncompartmental parameters such as: area under the curve (AUC), the time at which maximum concentration is reached (Tmax), the maximum concentration achieved (Cmax) and Lambda z, the rate constant associated with the terminal elimination phase.

The data was captured in spreadsheet format as was required for WinNonlin application (Appendix 2) for the various studies and consisted of the following information: subject code, schedule time when each blood specimen was taken, code for test or reference combination, code for the period when the combination was taken and concentration data for the respective drugs within the fixed drug combination as measured from the venous blood.

Making use of the schedule time information as well as the drug concentration levels, concentration-time curve plots for each of the series of drugs in a particular study were obtained (Appendix 3) using WinNonlin and analysed. The data derived from this analysis, together with the respective code information, allowed for the descriptive pharmacokinetic parameters, being, Tmax, Cmax, terminal half-life, area under time curve and area to infinity using the linear/log trapezoidal rule for the respective drugs to be generated. The logarithmic trapezoidal rule uses the linear trapezoidal calculation up to Cmax and the logarithmic trapezoidal rule is used after Cmax has been achieved. The parameters obtained were used to evaluate the bioavailability and bioequivalence of the FDC.

In all the studies, subjects served as their own control allowing the use of the paired t-test (parametric test) to statistically evaluate the comparison between the reference and the test. The t-test assumes that the population of differences follows a Gaussian distribution or normal distribution. Many statistical tests depend on the assumption that the values in the sample were obtained from a population that follows a Gaussian bell-shaped curve. As the normal distribution of the differences cannot be assumed, the non-parametric Wilcoxon matched pairs signed rank test was also used to confirm the results. The non-parametric tests make fewer assumptions about the distribution of the data. P-values less than 0.05 in the two – tailed test were considered to be significant. This analysis was determined in the widows-based GraphPad Prism® Version 2.01 (GraphPad Software© 1994,1995,1996 GraphPad Software, Inc).

For bioequivalence testing of the respective formulations the analysis was conducted according to the methods described by Hauschke et al [20]. The pharmacokinetic variables of principal importance are Cmax and AUC. Bioequivalence was declared if the 90% confidence interval for the test /reference ratio was completely within the range 80-125%, which is the acceptable standard set by the regulatory authority. Widows-based Microsoft ® Excel 97 SR-2 (Excel © 1985-1997 Microsoft Corporation) was used by formulating a programme in the application to determine the parameters as described by Hauschke. The graphics and other basic statistics were achieved using the windows based scientific graphing software programme SigmaPlot® version 2.01(SigmaPlot © 1986-1994 Jandel Corporation).

The procedure and process that was followed to investigate the minimum time points required to evaluate a given fixed dose combination (FDC) was as described above. The data analysis followed the order, rifampicin, isoniazid, pyrazinamide

and ethambutol, where one or more of the anti-tuberculosis drugs were present as reflected in Table 4, together with the expected therapeutic concentration range of the drugs concerned [27, 137, 146].

Table 4. Anti-tuberculosis drugs and recommended therapeutic range

Drug	Proposed 2 hour Cmax range (μg/ml)	Usual Dose (mg)
Rifampicin	8-24	600-750
Isoniazid	3-5	30-450
Pyrazinamide	20-60	1000-2000
Ethambutol	2-6	15-25

Table 5 reflects the time protocol schedule used with the extended time protocol that is currently used as policy (Phase1), the optimum time points (Phase 2) and the desired minimum time points (Phase 3) which were evaluated in this study.

Table 5. Extended, optimum and desired minimum time protocol schedule.

No. Time Points	Extended Time Points (hour) (PZA) Phase 1	Extended Time Points (hour) (RIF, INH, ETB) Phase 1	Optimum Time Points (hour) Phase 2	Desired Minimum Time Schedule (hour) Phase 3
1	0.00	0.00	0.00	0.00
2	0.25	0.25	0.25	1.00
3	0.50	0.50	0.50	2.00
4	1.00	1.00	1.00	4.00
5	1.50	1.50	1.50	6.00
6	2.00	2.00	2.00	8.00
7	2.50	2.50	2.50	
8	3.00	3.00	3.00	
9	4.00	4.00	4.00	
10	6.00	6.00	6.00	
11	8.00	8.00	8.00	
12	12.00	12.00		
13	24.00	24.00		
14	36.00			
15	48.00			

The time points chosen for phase 2 time protocol were based on the published work of Pillai et al [109] and Mcllleron et al [113] that concentrated on rifampicin in fixed dose combinations where time points up to 12 hours were used and 48 hours when pyrazinamide was included. Taking practical considerations into account when implementing clinical trials, 8 hours was used as the last sampling point in the investigation as the Tmax would have already been attained. The time points around the anticipated Cmax were retained to maintain accuracy. For the phase 3 time protocol the focus was placed on area under the time curve and not on Cmax.

The process that was implemented for the phase 2 and phase 3 analysis time protocol, as defined by the optimum and the desired minimum time schedule, respectively, was to de-select, from the initial set of data, the relevant time points from the extended protocol, to repeat the WinNonlin and Hauschke analysis for all the given drugs in the 13 studies and to evaluate if similar results and confidence intervals were achieved compared to that of the extended time protocol.

To determine the minimum number of subjects, the protocol followed the de-selecting of subjects from a selected four drug fixed dose combination study. The outcome had to achieve similar results and confidence intervals to that of the extended time protocol to be successful and verify the minimum number.

Sub-group analysis and correlation analysis were investigated for all 13 studies as a collective data set. Comparisons for the collective data set were derived for the single reference principal versus that of the active principal in fixed combination, the influence of sex and that of disease status. The same application and statistical methods were applied as in the investigations for the

optimum minimum time points. Where this was not possible based on unpaired data sets, due to subjects not completing both arms of a given study, the Mann-Whitney non –parametric test was applied. Linear mixed models were fitted to the logarithm of the difference between the AUCT parameters [148, 176]. The residual maximum likelihood algorithm (REML) of the GenStat © 2002 6th edition computer package was used for the fitting as opposed to the standard maximum likelihood method. The standard maximum likelihood method estimates of variance components tend to be underestimated, because they do not take into account the loss of degrees of freedom due to the estimation of fixed effects. The REML is intended to solve this problem.

Chapter 3
Extended Time Protocol Results

3.1 Bioavailability and Bioequivalence

A short outline describing each of the studies investigated is presented. Figures 2 to 15 and Tables 6 to Table 19 and Table 20 present the results of the studies and summarises the bioavailability and bioequivalence analysis with respect to these studies. The tables present the data in reference and test formulations of the pharmacokinetic parameters Tmax, Cmax, half-life, AUCT and AUCI including the mean and standard error of the mean (SEM). The units of measurement for each of the parameters are indicated in brackets; Tmax (h), Cmax (mg/l), Half–life (h), AUC and AUCI (mg.h/l). The parametric Students–t-Test and non-parametric Wilcoxon Signed Rank test were used to determine statistical significance between the reference and test formulations. Significance was declared where the p – values obtained were less than 0.05 when comparing the reference to the test. For bioavailability to be successful, for both the reference or test of each of the respective drugs, Cmax should be within the therapeutic range as defined in Table 4.

For bioequivalence testing, the parameters of importance, i.e. Cmax, AUC and AUCI were summarised with respect to the point estimate, test/ reference ratio, the percentage span (the difference between the upper and lower confidence interval), and the most important parameter in the assessment, the 90% confidence interval of the respective drugs. Where the data falls within the confidence interval (80-125%) the data is presented in bold and when not within the required parameters, presented as ordinary text. An explanation and conclusion of each of the studies are provided to the figures and tables of the respective studies in the following order: Cmax, AUC and AUCI. Tmax and half-

life data are presented as part of the graphics but the general and overall conclusion across all studies is that Tmax and half-life were comparable with some exceptions in Tmax mainly due to the nature of the formulation relative to the reference.

Study 1

The objective of the study was to compare the bioavailability, in healthy volunteers, of rifampicin and isoniazid in fixed combination formulation tablets Rifinah 150 and Rifinah 300, to the bioavailability of the reference single agents, rifampicin and isoniazid, currently registered in South Africa for use in tuberculosis therapy. Data from this study is summarised in Fig 2 and 3 and Table 6 and 7 taking into consideration both components of the respective studies. Study 1.1 (Rifinah 150) and Study 1.2 (Rifinah 300) investigated two different dosages relative to a reference in a three way cross-over design with subjects randomised in period one and period three of the study and receiving the reference in period two. For the bioequivalence analysis, two assumptions are made: (1) that subjects received the reference drug in period one and the test drug in period two (2) for purposes of the analysis the first eight subjects received the drug in the order of reference then test and the second eight received it in the reverse order.

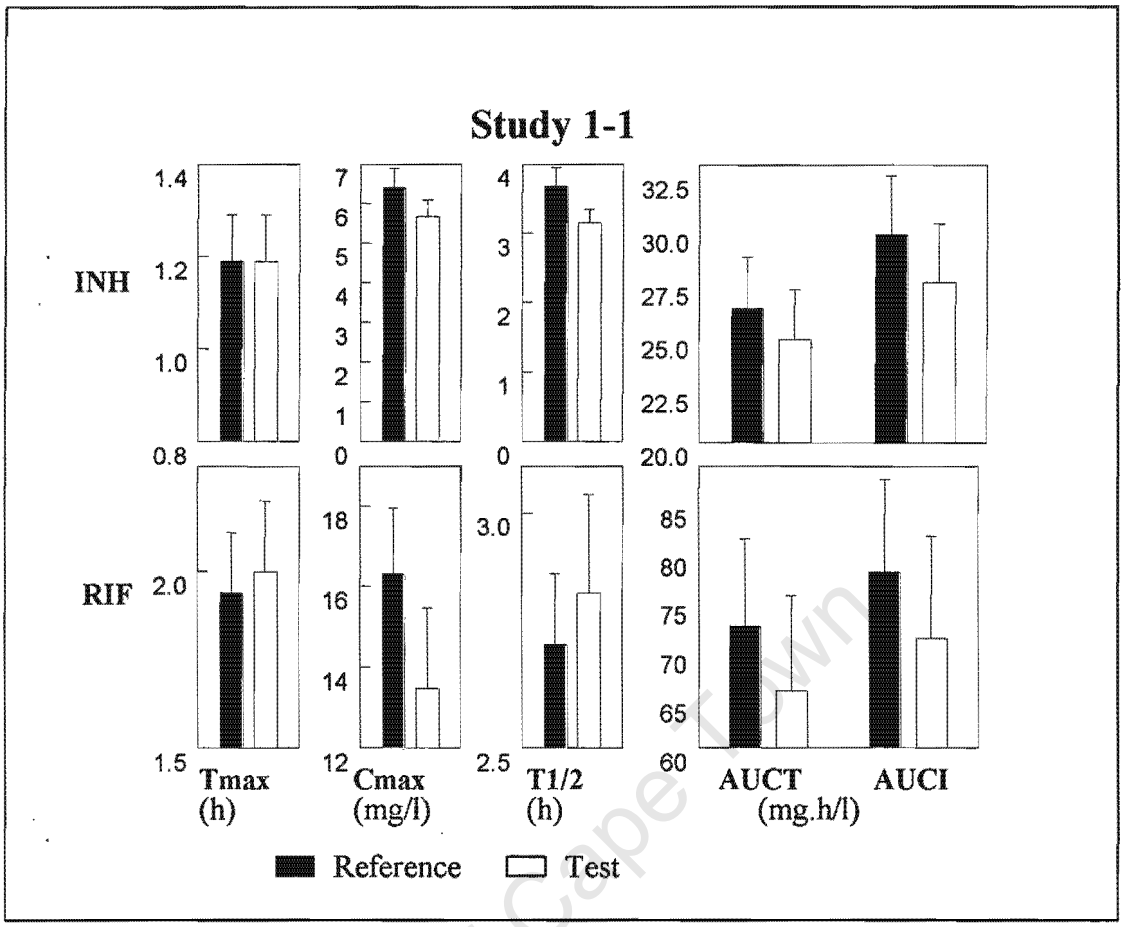


Fig 2. Bioavailability results of study 1.1

Table 6. Bioequivalence results of study 1.1

Study 1.1 N=16 HV	Median/ Point Estimate	Test/ Reference Ratio	90% Confidence Interval	% Span
Isoniazid (INH)				
Cmax	95.52	0.94	**81.61-112.56**	30.95
AUC	100.68	0.97	**87.88-116.11**	28.23
AUCI	103.71	0.95	**89.17-120.17**	30.99
Rifampicin (RIF)				
Cmax	94.38	0.99	62.46-144.96	82.50
AUC	94.10	1.07	68.43-138.95	70.52
AUCI	95.59	1.04	68.17-133.42	65.25

Both Study 1.1 and Study 1.2 concluded bioavailability based on Cmax, AUCT and AUCI, with no significant difference to the reference. Bioequivalence was concluded within the confidence interval limits for INH in both study 1.1 and study 1.2 for parameters Cmax, AUCT and AUCI, but not for RIF. The product formulation was successful based on the assessment of bioavailability data only indicating that both drugs were bioavailable although not bioequivalent.

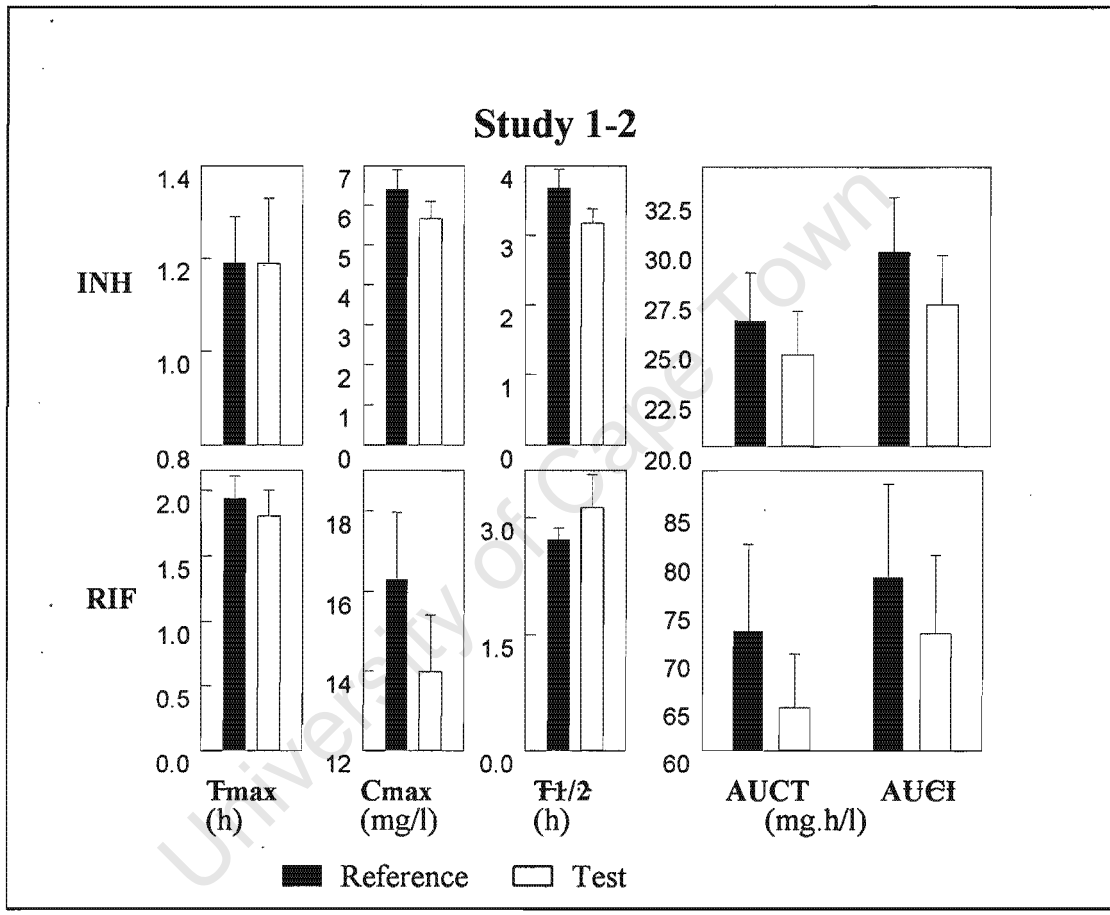


Fig 3. Bioavailability results of study 1.2

Table 7. Bioequivalence results of study 1.2

Study 1.2 N=16 HV	Median/ Point Estimate	Test/ Reference Ratio	90% Confidence Interval	% Span
Isoniazid (INH)				
Cmax	106.59	0.92	**93.11-123.76**	30.64
AUC	102.04	0.96	**91.88-114.33**	22.45
AUCI	110.50	0.94	**96.12-123.65**	27.53
Rifampicin (RIF)				
Cmax	88.16	1.01	69.31-119.74	50.43
AUC	94.76	1.06	72.70-133.29	60.59
AUCI	102.09	1.06	87.90-139.66	51.77

Study 2

The first objective of the study was to compare the bioavailability, in healthy, volunteers of a Myrin-P fixed combination tablet consisting of rifampicin, isoniazid, pyrazinamide and ethambutol as a single dose to that of the reference tablets of rifampicin (capsule), isoniazid, pyrazinamide and ethambutol tablets as single doses. The second objective was to establish whether the Myrin-P fixed combination formulation is bioequivalent to equivalent doses of the drugs in the reference products.

The study (Fig 4) demonstrated bioavailability based on Cmax in INH, RIF, PZA and ETB with no significant difference when comparing the test product to that of the reference with the same drug constituents. AUCT and AUCI showed no significant difference in RIF and ETB but did for INH. PZA, in test and reference was not significantly different in AUCI but in AUCT. The bioequivalence analysis (Table 8) concluded that the parameters Cmax, AUCT and AUCI for INH , RIF and PZA were within the required confidence limit including

that for Cmax of ETB. Based on AUC and AUCI ETB showed bio-inequivalence. The product formulation was declared successful based on the bioavailability and bioequivalence assessment.

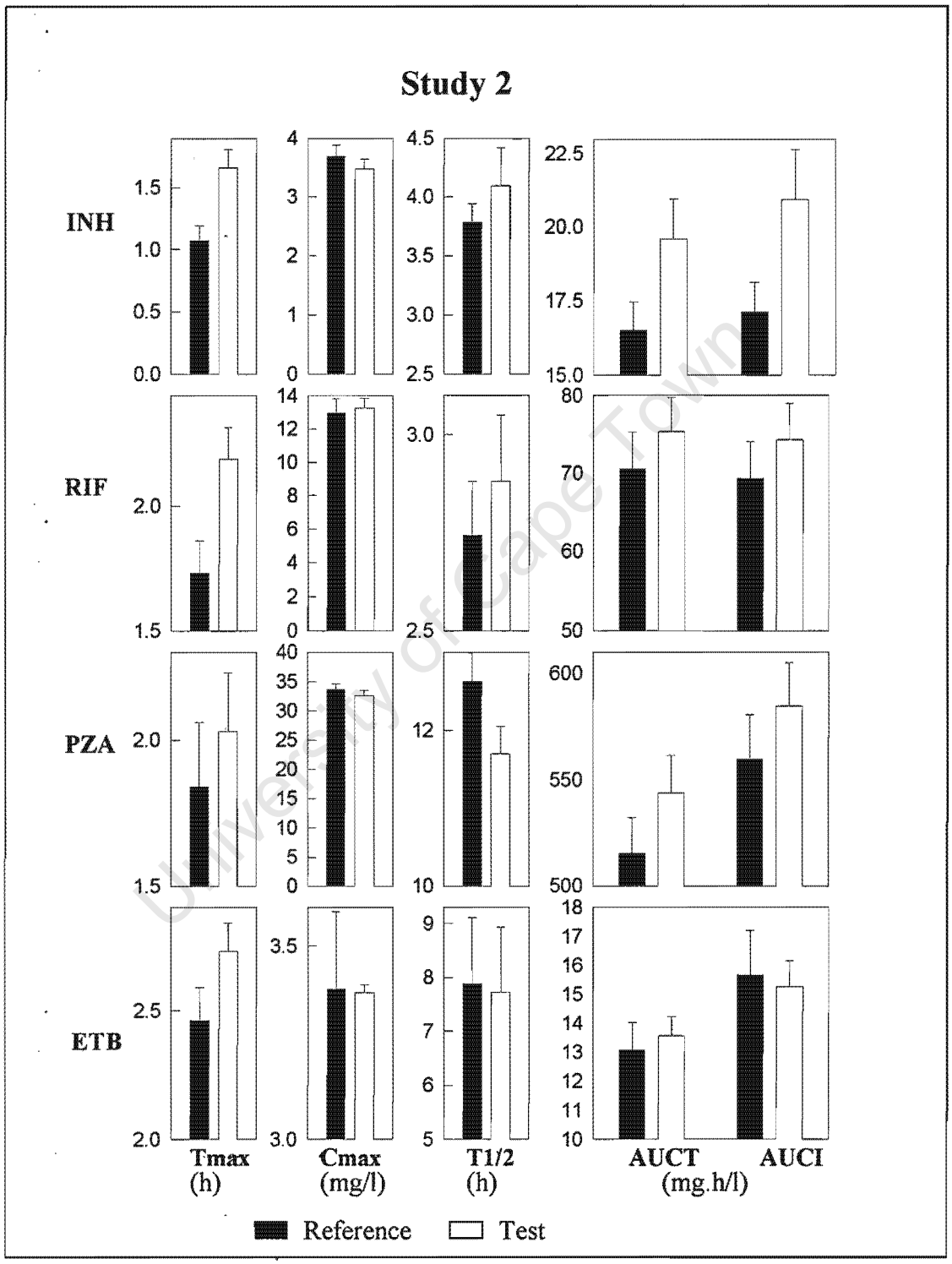


Fig 4. Bioavailability results of study 2

Table 8. Bioequivalence results of study 2

Study 2 N=24 HV	Median/ Point Estimate	Test/ Reference Ratio	90% Confidence Interval	% Span
Isoniazid (INH)				
Cmax	94.46	1.02	**83.17-105.29**	22.12
AUC	116.23	1.19	108.76-126.30	17.54
AUCI	113.29	1.17	**106.31-120.07**	13.76
Rifampicin (RIF)				
Cmax	104.18	1.08	**95.30-114.10**	18.79
AUC	108.22	1.10	**100.15-116.49**	16.34
AUCI	108.27	1.10	**99.77-115.82**	16.05
Pyrazinamide (PZA)				
Cmax	95.90	0.98	**91.10-100.14**	9.04
AUC	105.75	1.06	**103.11-108.59**	5.49
AUCI	95.00	1.05	**93.27-97.87**	4.59
Ethambutol (ETB)				
Cmax	97.15	1.08	**84.27-114.70**	30.42
AUC	106.03	1.17	90.12-125.05	34.94
AUCI	108.78	1.18	87.23-136.29	49.06

Study 3

The objective of the study was to compare the bioavailability, in patients, of rifampicin, isoniazid and pyrazinamide for the fixed drug combination of Rifcin PedZ tablets to the bioavailability of the reference single agent products, rifampicin (capsules), isoniazid (tablets) and pyrazinamide (tablets) currently registered in South Africa for the treatment of tuberculosis.

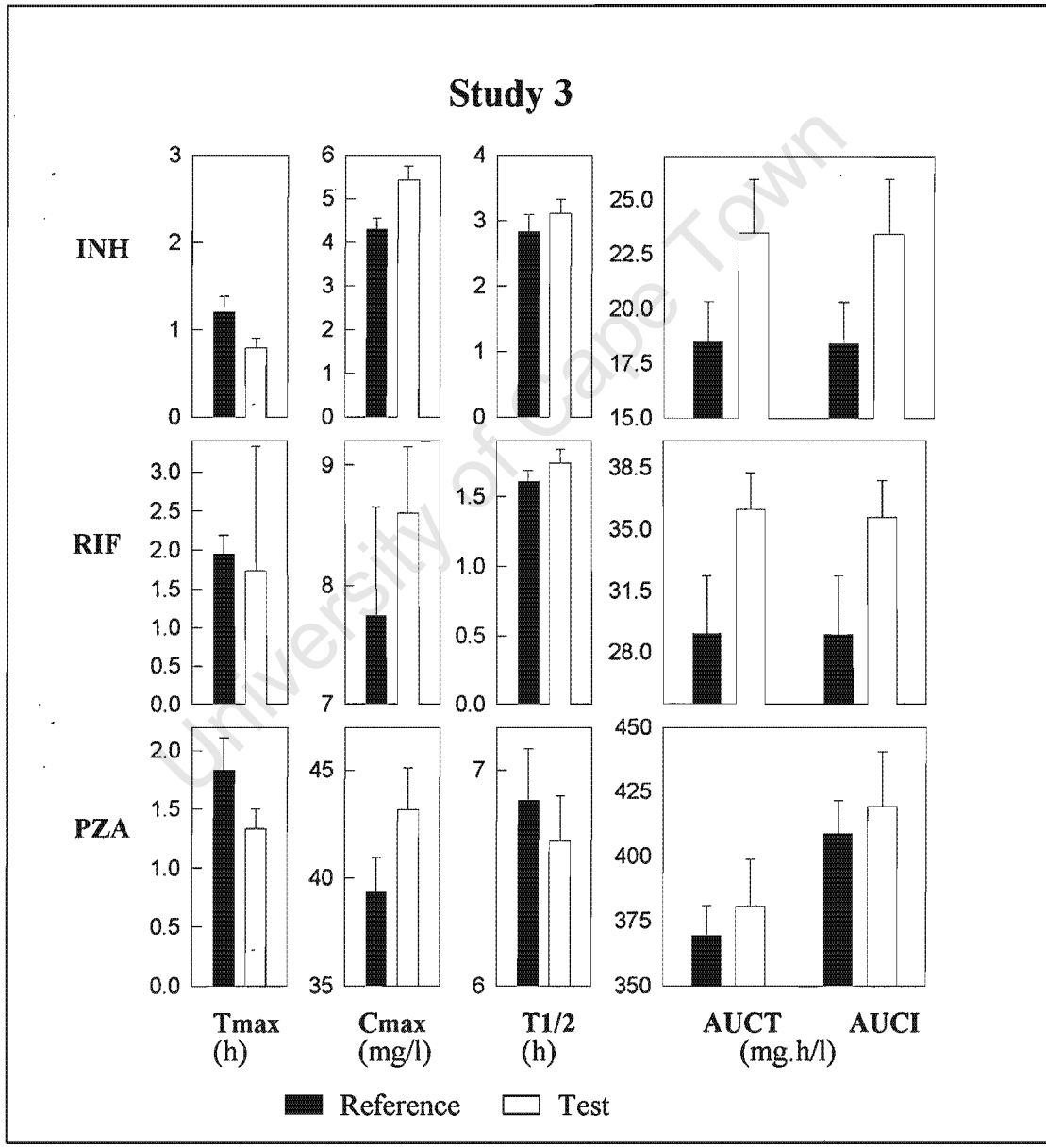


Fig 5. Bioavailability results of study 3

The study concluded (Fig 5) that adequate bioavailability of all three drugs was achieved and comparable to the reference. Bioavailability was achieved for Cmax that was not significantly different for RIF and PZA but that of INH was significantly different. AUCT and AUCI showed partial significantly different results for INH. RIF showed a significant difference for AUCT and AUCI and PZA showed no significant difference for the same parameters with respect to bioavailability.

Table 9. Bioequivalence results of study 3

Study 3 N=16 Patients	Median/ Point Estimate	Test/ Reference Ratio	90% Confidence Interval	% Span
Isoniazid (INH)				
Cmax	126.06	1.35	101.62-148.53	46.91
AUC	121.95	1.53	100.87-144.48	43.61
AUCI	122.81	1.54	**104.79-122.81**	44.67
Rifampicin (RIF)				
Cmax	111.32	1.20	89.10-143.41	54.31
AUC	127.08	1.32	109.72-154.01	44.29
AUCI	125.88	1.32	109.34-155.36	46.02
Pyrazinamide (PZA)				
Cmax	108.22	1.07	**96.97-118.82**	21.85
AUC	102.01	1.00	**91.48-108.08**	16.60
AUCI	101.01	0.99	**89.12-106.12**	17.01

Bioequivalence as presented by Table 9 was shown completely for PZA and only for AUCI for INH. RIF did not fall within the desired confidence interval as required for all parameters of Cmax, AUCT and AUCI. The product was successful based on bioavailability falling within limits with respect to Cmax although the bioequivalence was unsuccessful.

Study 4

The study objective was to compare, in patients, the bioavailability of rifampicin, isoniazid and pyrazinamide in the fixed drug combination of Rifcin Co tablets to the bioavailability achieved by the reference single agents, rifampicin (capsules), isoniazid (tablets) and pyrazinamide (tablets) currently registered in South Africa for the treatment of tuberculosis.

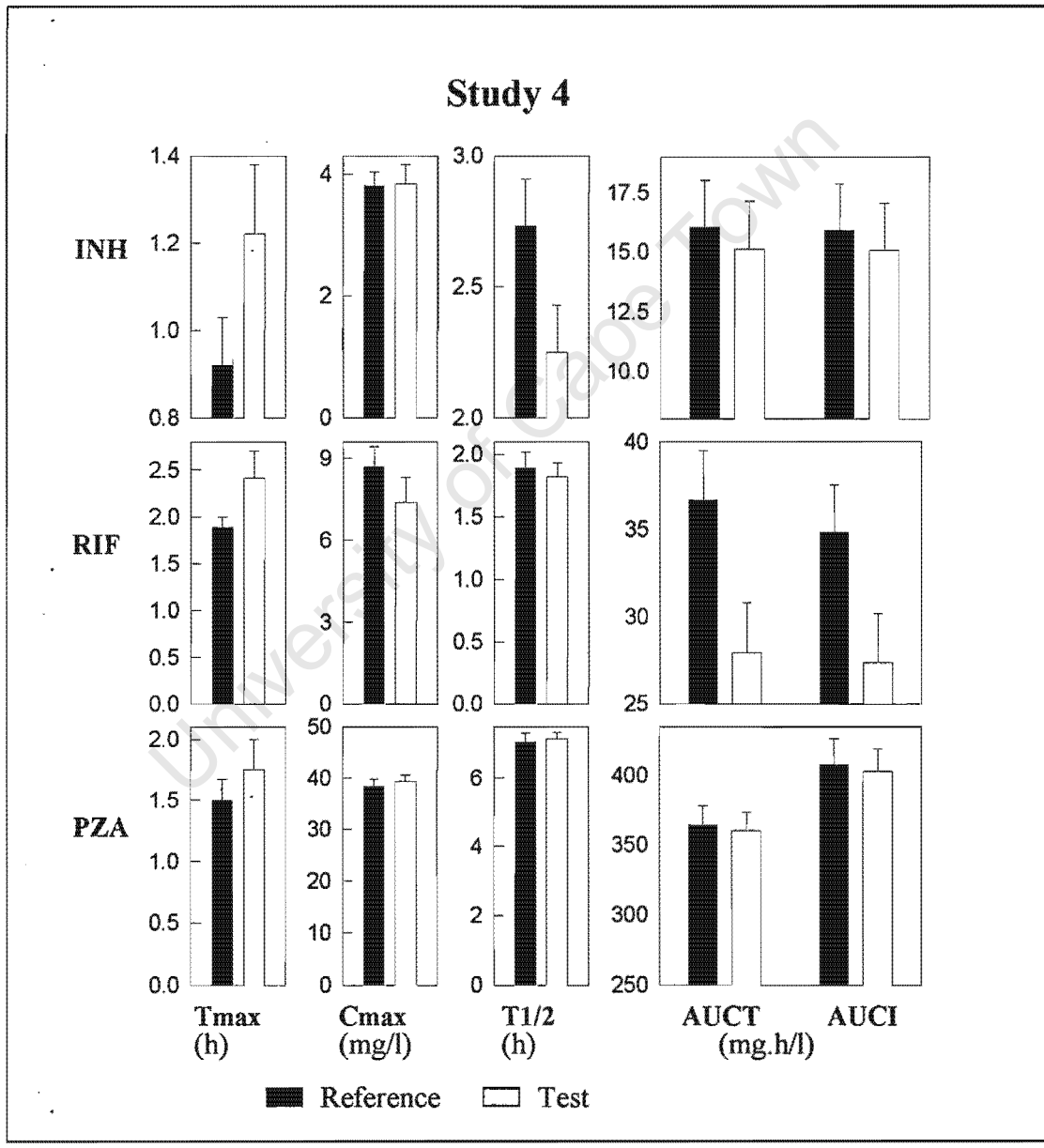


Fig 6. Bioavailability results of study 4

The study, as indicated by Fig 6 concluded bioavailability for Cmax with respect to all three drugs that was not significantly different to that of the reference. AUCT and AUCI for both INH and PZA showed no significant difference, but RIF showed significant difference for AUCT and a partial significant difference for AUCI.

Table 10. Bioequivalence results of study 4

Study 4 N=22 Patients	**Median/ Point Estimate**	**Test/ Reference Ratio**	**90% Confidence Interval**	**% Span**
Isoniazid (INH)				
Cmax	95.62	1.05	**80.43-113.37**	32.94
AUC	88.14	0.97	**79.73-111.05**	31.32
AUCI	89.28	0.98	**80.78-110.67**	29.89
Rifampicin (RIF)				
Cmax	88.95	0.95	70.31-111.35	41.04
AUC	84.30	0.90	67.03-108.69	41.66
AUCI	84.48	0.89	67.21-108.91	41.70
Pyrazinamide (PZA)				
Cmax	104.45	1.04	**96.09-111.16**	15.07
AUC	98.89	0.99	**95.80-102.46**	6.66
AUCI	99.72	1.00	**95.82-103.22**	7.40

Bioequivalence as presented by Table 10 was within the 90% confidence interval for all parameters for INH and PZA but not for RIF. Adequate bioavailability is shown for the formulation with respect to Cmax, but based on the bio-inequivalence of RIF renders the product formulation unsuccessful.

Study 5

The objective of the study was to compare the bioavailability in patients of rifampicin and isoniazid, in fixed drug combination Rifcin Ped tablets, to the bioavailability achieved by the single reference agents of rifampicin (capsules) and isoniazid (tablets), currently registered in South Africa for treatment of tuberculosis.

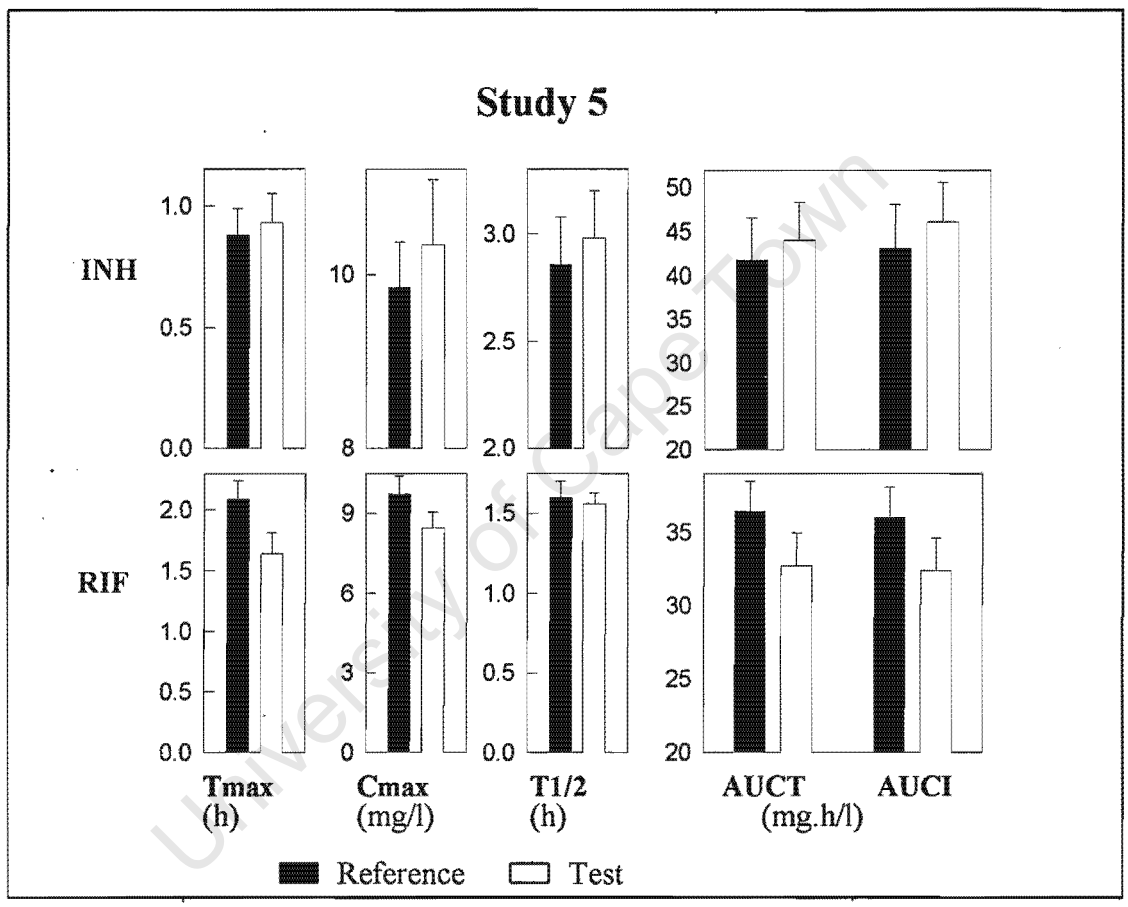


Fig 7. Bioavailability results of study 5

The study as summarised in Fig 7 showed that bioavailability for both INH and RIF for all the pharmacokinetic parameters investigated was successfully achieved in both test and reference formulations.

Table 11. Bioequivalence results of study 5

Study 5 N=19 Patients	Median/ Point Estimate	Test/ Reference Ratio	90% Confidence Interval	% Span
Isoniazid (INH)				
Cmax	98.35	1.09	**87.46-114.80**	27.34
AUC	104.12	1.14	**95.16-118.54**	23.38
AUCI	105.07	1.15	**96.24-120.51**	24.27
Rifampicin (RIF)				
Cmax	90.69	0.97	77.32-109.09	31.77
AUC	94.37	0.97	**84.42-106.20**	21.78
AUCI	94.22	0.98	**84.53-108.62**	24.09

Bioequivalence analysis as presented in Table 11 concluded that AUCT and AUCI was within the desired confidence interval for both INH and RIF. Bioequivalence for Cmax was successful for INH, but not for RIF that was outside the confidence interval. The product formulation was successfully achieved based on bioavailability and bioequivalence data, of AUC and AUCI.

Study 6

The objective of the study was to compare the bioavailability in healthy volunteers of rifampicin for Rifadin tablet to that of rifampicin in the registered drug rifampicin Rimactane (tablet) as reference and the second objective was to establish whether the two formulations were bioequivalent, after a single dose.

The study concluded bioavailability as presented in Fig 8 for RIF for all parameters of Cmax, AUC and AUCI compared to that of the reference formulation investigated. Bioequivalence as presented in Table 12 was shown for

Cmax, AUC and AUCI. The product formulation was successful based on bioavailability and bioequivalence.

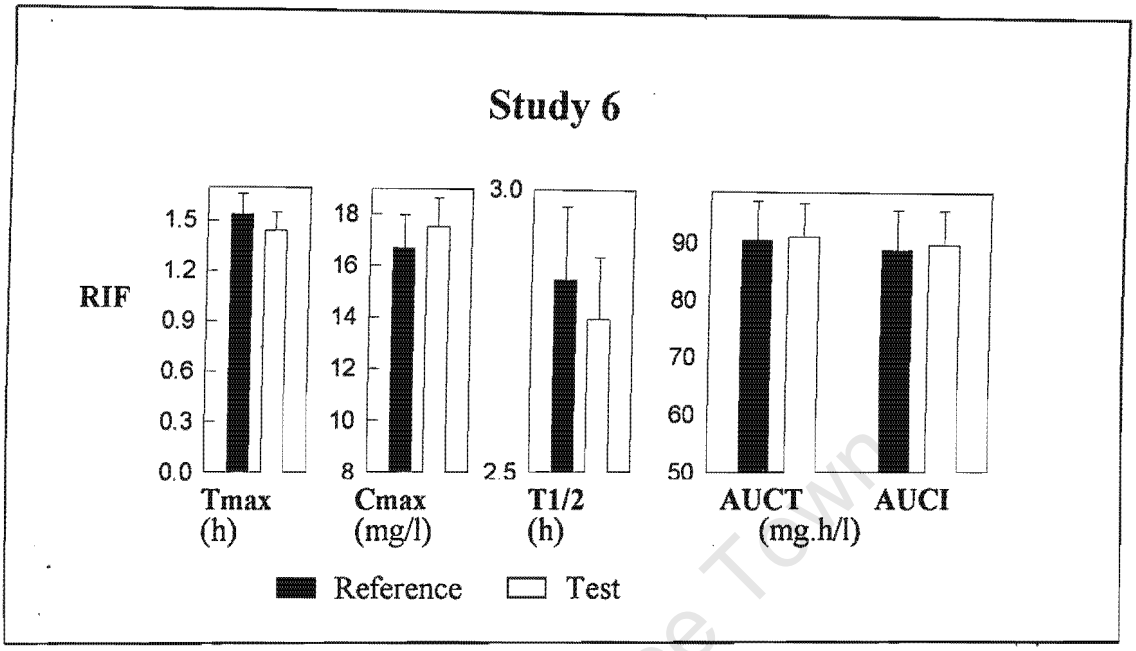


Fig 8. Bioavailability results of study 6

Table 12. Bioequivalence results of study 6

Study 6 N=24 HV.	Median/ Point Estimate	Test/ Reference Ratio	90% Confidence Interval	% Span
Rifampicin (RIF)				
Cmax	108.62	1.08	**96.29-119.42**	23.13
AUC	103.60	1.03	**98.62-109.16**	10.53
AUCI	103.61	1.04	**98.54-109.49**	10.94

Study 7

The objective of study was to compare the bioavailability in healthy volunteers of Rifafour fixed combination tablets as a single dose, consisting of rifampicin, isoniazid , pyrazinamide and ethambutol to that of the single reference agents of rifampicin, isoniazid, pyrazinamide and ethambutol tablets in single doses. The second objective was to establish whether the fixed combination formulation, Rifafour, is bioequivalent to equivalent doses of the same reference products.

The study found, as demonstrated in Fig 9, that bioavailability was achieved for the parameters Cmax, AUCT and AUCI for all four drugs, in the fixed-dose drug combination with no significant difference to the respective reference. Bioequivalence as presented by Table 13 complied within the desired 90% confidence interval for all parameters of Cmax, AUCT and AUCI for all the drugs. The product formulation was declared successful based on bioavailability and bioequivalence data.

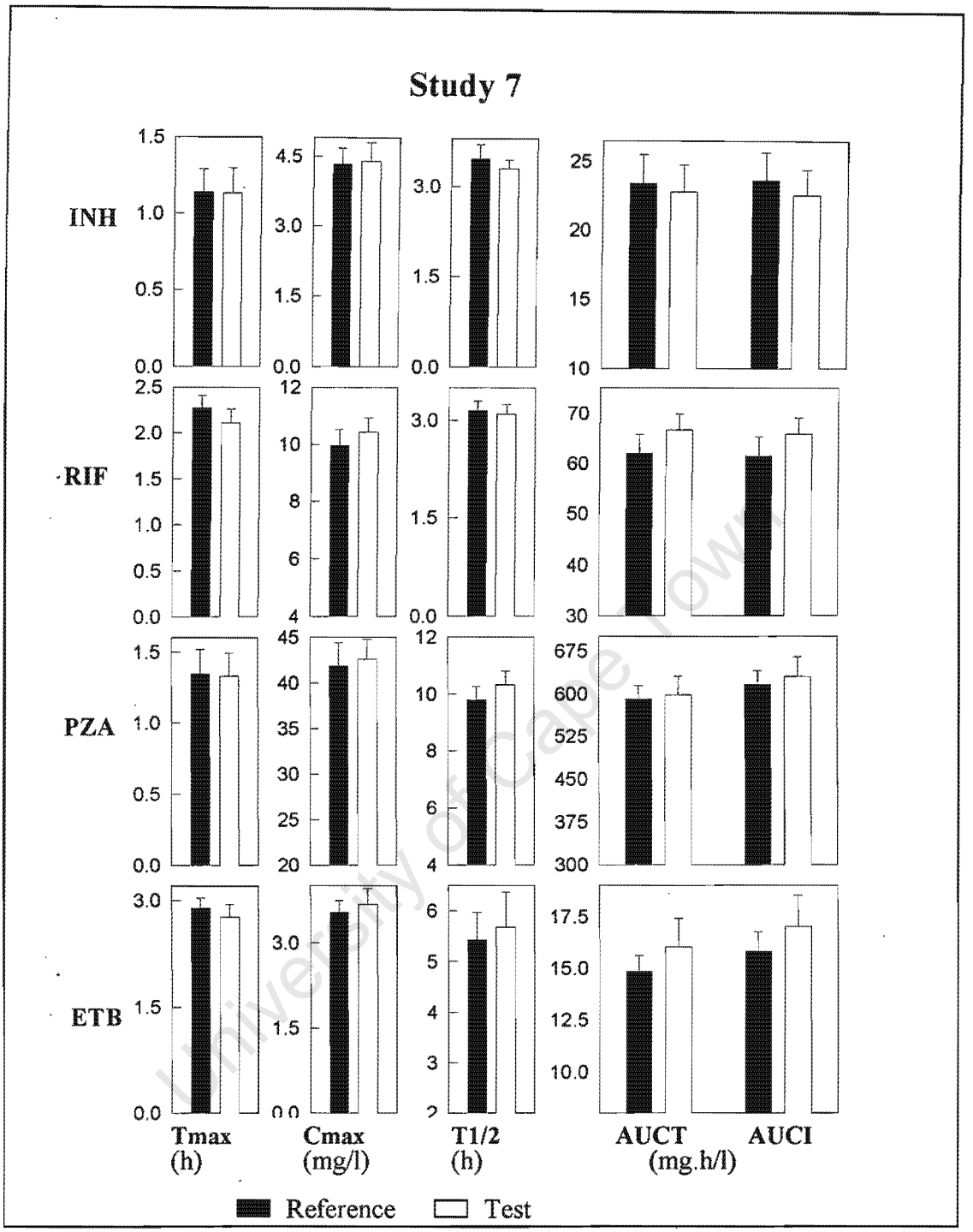


Fig 9. Bioavailability results of study 7

Table 13. Bioequivalence results of study 7

Study 7 N=23 HV	Median/ Point Estimate	Test/ Reference Ratio	90% Confidence Interval	% Span
Isoniazid (INH)				
Cmax	100.86	1.06	**89.80-115.33**	25.53
AUC	95.47	1.04	**89.16-107.76**	18.61
AUCI	92.54	1.01	**87.09-105.77**	18.68
Rifampicin (RIF)				
Cmax	101.82	1.12	**92.87-119.06**	26.19
AUC	105.98	1.13	**98.02-115.01**	16.99
AUCI	107.77	1.11	**97.54 -115.50**	17.96
Pyrazinamide (PZA)				
Cmax	104.24	1.04	**100.32-110.97**	10.65
AUC	98.77	1.01	**95.68-103.24**	7.57
AUCI	99.41	1.02	**95.84-105.61**	9.77
Ethambutol (ETB)				
Cmax	100.96	1.06	**89.85-114.06**	24.20
AUC	100.44	1.09	**90.90-113.45**	22.55
AUCI	98.71	1.10	**88.12-113.35**	25.22

Study 8

The objective of the study was to do a blinded study to compare the bioavailability of rifampicin and its metabolite desacetyl rifampicin, in a single fixed-dose combination tablet to a single reference agent. The bioavailability of a fixed-dose combination tablet(WHO), consisting of rifampicin, isoniazid, pyrazinamide was compared to that of the single reference agents as a single dose in healthy volunteers. The second objective was to establish whether the fixed combination

formulation, "WHO", is bioequivalent to equivalent doses of the same reference products.

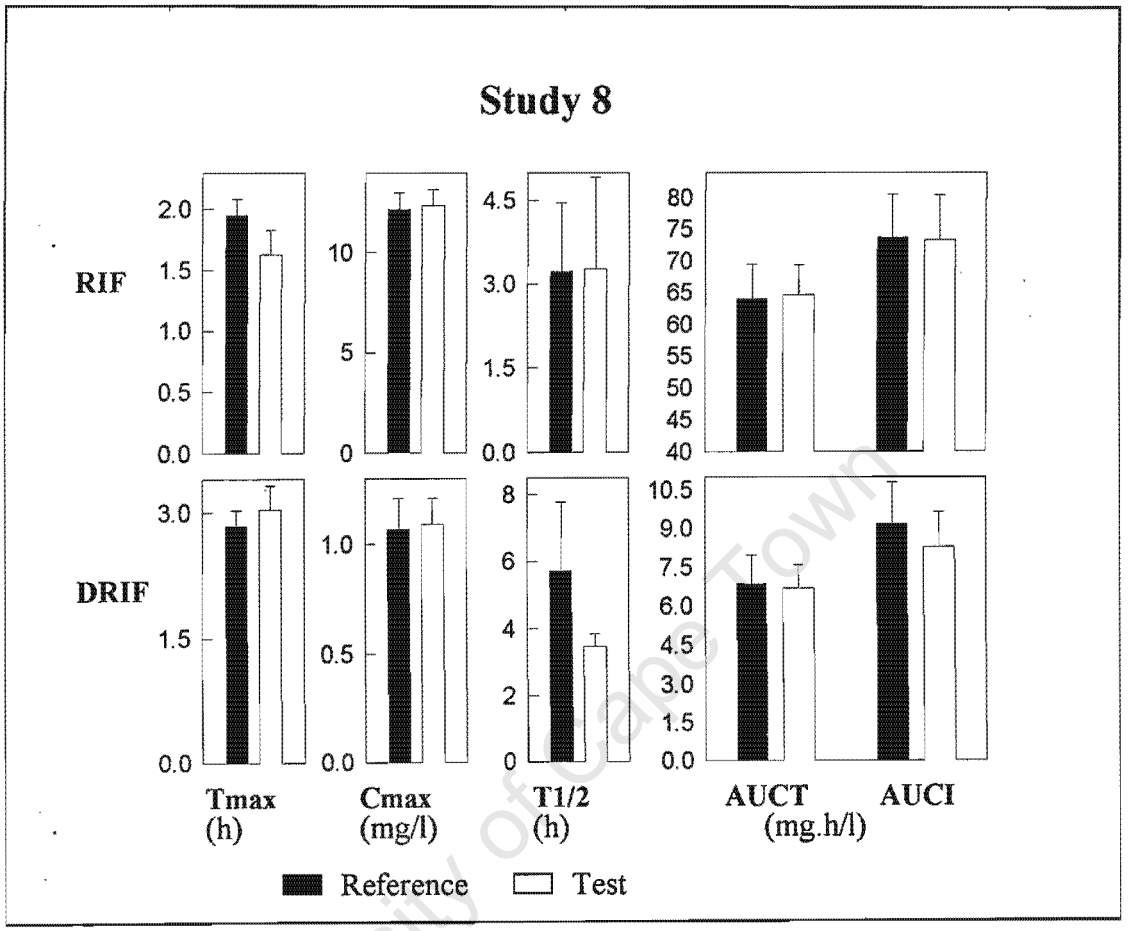


Fig 10. Bioavailability results of study 8

Table 14. Bioequivalence results of study 8

Study 8 N=19 HV	Median/ Point Estimate	Test/ Reference Ratio	90% Confidence Interval	% Span
Rifampicin (RIF)				
Cmax	100.48	1.06	**89.21-115.19**	25.98
AUC	101.11	1.06	**92.20-112.26**	20.07
AUCI	100.23	1.04	**92.85-110.72**	17.87
Des Rifampicin (DRIF)				
Cmax	108.71	1.12	**93.09-122.47**	29.38
AUC	100.88	1.11	**87.02-120.87**	33.85
AUCI	93.22	1.06	**83.56-112.79**	29.22

The study assayed only RIF and DRIF and as indicated in Fig 10, and demonstrated bioavailability within the required range for RIF and the metabolite DRIF, for parameters Cmax, AUCT and AUCI that was not significantly different from the reference.

Bioequivalence testing using Hauschke analysis was satisfactorily achieved as presented by Table 14 that fell within the confidence interval as required for Cmax, AUCT and AUCI. The product formulation was declared successful based on bioavailability and bioequivalence data.

Study 9

The objective of the study was to compare the bioavailability in healthy volunteers of Rifinah Combination Paediatric Powder sachets as a single dose, consisting of rifampicin and isoniazid, to that of the reference drugs rifampicin and isoniazid as single dose tablets. The second objective was to establish whether

the formulation Rifinah Combination Paediatric Powder is bioequivalent to equivalent doses of the drugs in the reference product.

The conclusion of the study (Fig 11) demonstrated bioavailability that was significantly different for Cmax, relative to the respective reference drugs, for INH and RIF respectively. Bioavailability parameters AUCT and AUCI for INH showed no significant difference, but AUCT and AUCI for RIF were significantly different when comparing the test to reference formulations.

Bioequivalence analysis as presented by Table 15 was satisfactory for AUC and AUCI for INH, but not for Cmax for the same drug. RIF showed bio-inequivalence for AUCT , AUCI and Cmax. The product formulation was bioavailable within the required limits but the test product was declared unsuccessful due to the bioequivalence parameters not falling within the confidence interval as required.

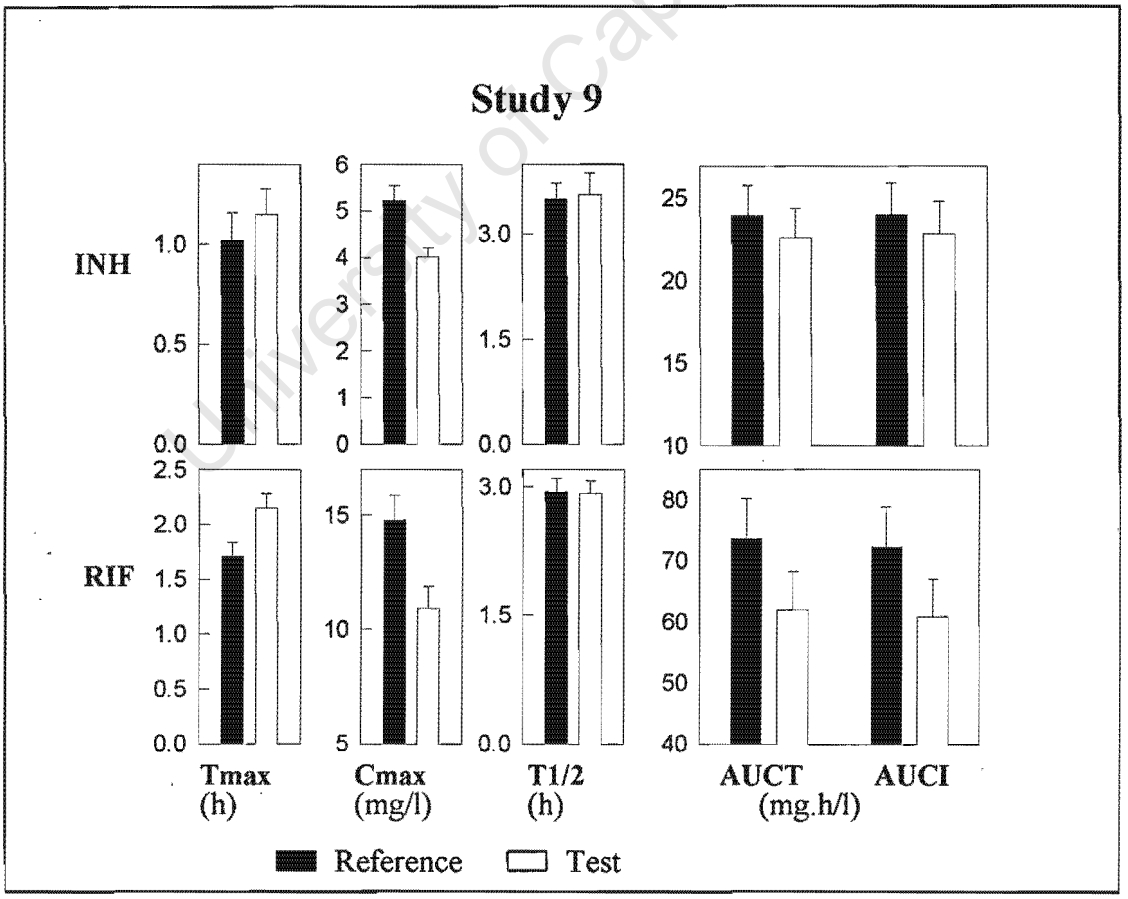


Fig 11. Bioavailability results of study 9

Table 15. Bioequivalence results of study 9

Study 9 N=24 HV	Median/ Point Estimate	Test/ Reference Ratio	90% Confidence Interval	% Span
Isoniazid (INH)				
Cmax	78.93	0.83	69.89-88.58	18.69
AUC	94.29	1.00	**82.20-106.66**	24.46
AUCI	96.05	1.01	**81.95-108.88**	26.92
Rifampicin (RIF)				
Cmax	74.43	0.76	67.37-82.88	15.51
AUC	83.65	0.85	76.47-92.35	15.88
AUCI	84.09	0.85	76.21-92.97	16.76

Study 10

The objective of the study was to compare the bioavailability in healthy volunteers of Rifater Combination Paediatric Powder sachets as fixed-dose combination, consisting of rifampicin, isoniazid and pyrazinamide to that of the reference individual drugs, rifampicin, isoniazid and pyrazinamide as single dose tablets. The second objective was to establish whether the formulation Rifater Combination Paediatric Powder sachets is bioequivalent to equivalent doses of the drugs in the reference products.

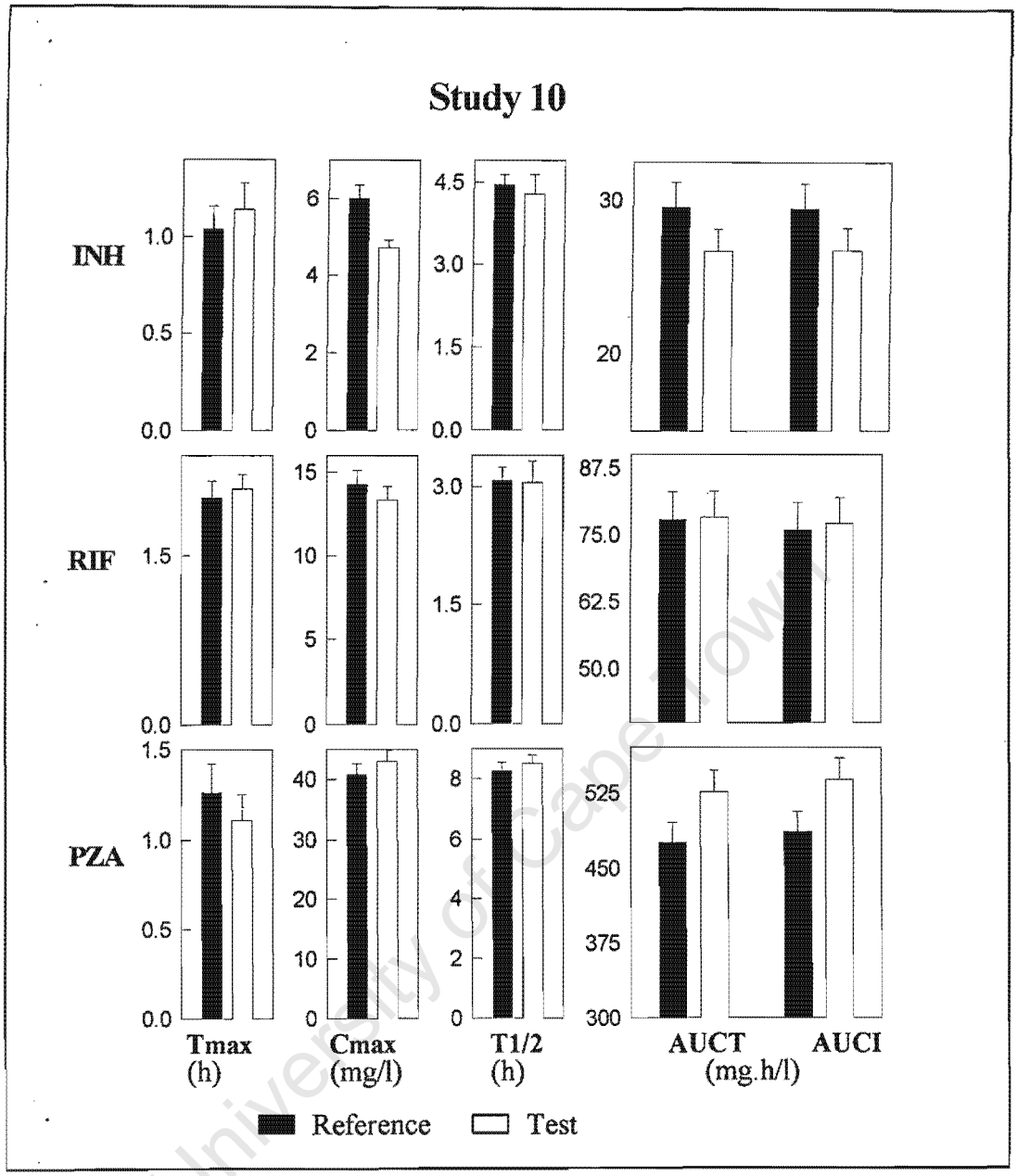


Fig 12. Bioavailability results of study 10

The study concluded as indicated in Fig 12, showed that Cmax for both RIF and PZA respectively was bioavailable and also not significantly different relative to their respective reference products. INH was bioavailable, but significantly different to that of the reference product. AUCT and AUCI for RIF were not significantly different, while for INH and PZA, in the test product, these parameters were significantly different relative to the reference product.

Table 16. Bioequivalence results of study 10

Study 10 N=21 HV	Median/ Point Estimate	Test/ Reference Ratio	90% Confidence Interval	% Span
Isoniazid (INH)				
Cmax	79.77	0.81	73.37-83.75	10.38
AUC	93.01	0.93	**86.64-97.70**	11.05
AUCI	91.16	0.92	**85.64-96.33**	10.69
Rifampicin (RIF)				
Cmax	88.04	1.01	**81.38-104.10**	22.72
AUC	97.21	1.06	**87.70-112.29**	24.59
AUCI	96.48	1.07	**88.59-112.43**	23.84
Pyrazinamide (PZA)				
Cmax	104.41	1.08	**97.69-113.47**	15.78
AUC	110.96	1.11	**106.86-115.05**	8.18
AUCI	111.08	1.11	**107.51-114.80**	7.29

The bioequivalence results summarised in Table 16 were satisfactory for the parameters Cmax, AUCT and AUCI for RIF and PZA and for INH only AUCT and AUCI complied. The product formulation was successful based on bioequivalence.

Study 11

The objective of the study was to compare the bioavailability in healthy volunteers of Rifinah 150/150 fixed-dose combination tablets, consisting of rifampicin and isoniazid as a single dose to the reference tablets of rifampicin and isoniazid as a single dose. The second objective was to establish whether the fixed-dose combination formulation Rifinah 150/150 fixed combination tablets is bioequivalent to equivalent doses of the drugs in the reference products.

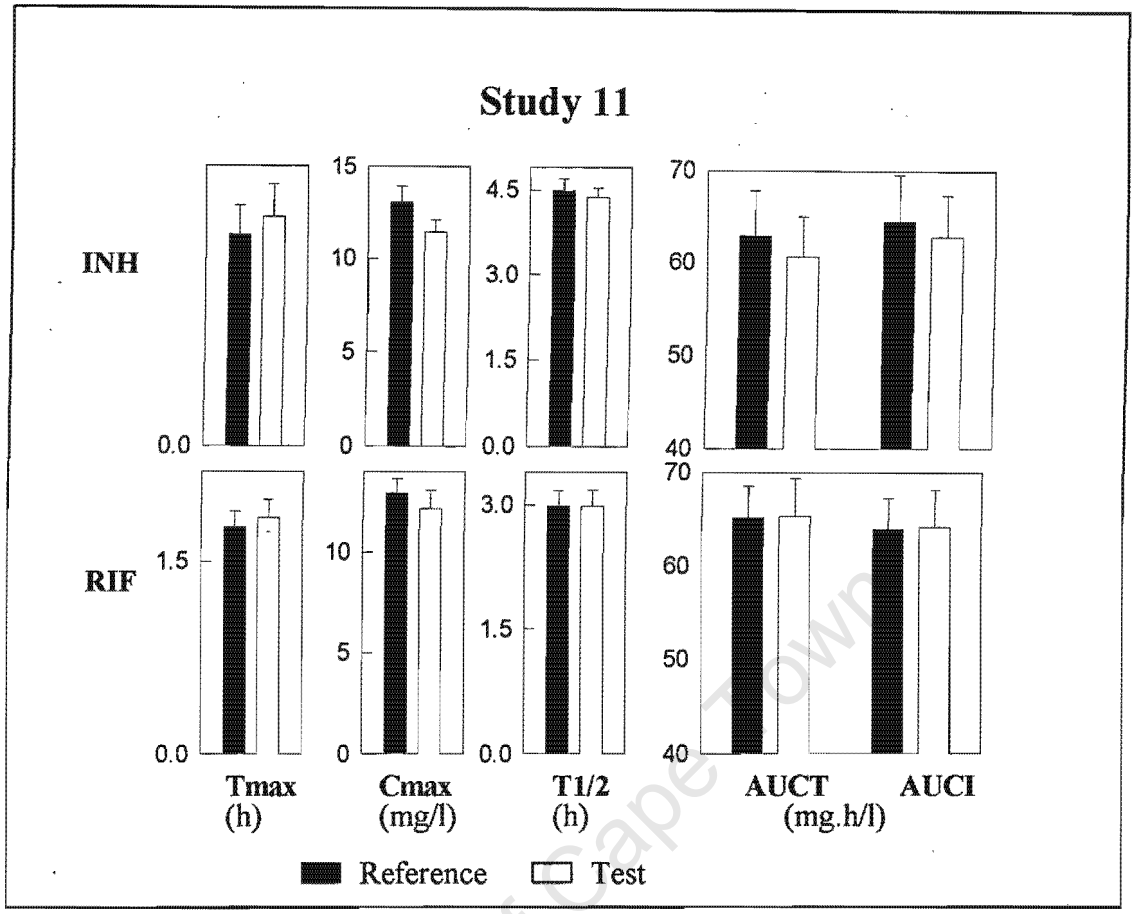


Fig 13. Bioavailability results of study 11

The study concluded (Fig 13) that INH and RIF were bioavailable in reference and test based on the parameters Cmax, AUCT and AUCI. The comparison of the test to reference products respectively showed no-significant difference.

Bioequivalence analysis presented in Table 17 was adequately achieved for both INH and RIF for the analysis parameters Cmax, AUCT and AUCI. The product formulation was declared successful based on both bioavailability and bioequivalence data.

Table 17. Bioequivalence results of study 11

Study 11 N=22 HV	Median/ Point Estimate	Test/ Reference Ratio	90% Confidence Interval	% Span
Isoniazid (INH)				
Cmax	98.79	0.93	**85.18-111.62**	26.44
AUC	102.07	1.02	**93.95-114.50**	20.55
AUCI	102.03	1.03	**93.54-115.91**	22.36
Rifampicin (RIF)				
Cmax	95.61	0.97	**86.21-106.46**	20.25
AUC	97.96	1.02	**90.36-109.96**	19.60
AUCI	97.33	1.03	**89.11-109.10**	19.99

Study 12

The objective of the study was to compare the bioavailability in healthy volunteers of Rifinah 150/75 fixed combination tablets consisting of rifampicin and isoniazid as a single dose to the reference tablets of rifampicin and isoniazid as a single dose. The second objective was to establish whether the fixed dose combination formulation Rifinah 150/75 fixed combination tablets is bioequivalent to equivalent doses of the drugs in the reference products.

The study concluded as indicated in Fig 14 that INH was bioavailable with respect to Cmax with no significant statistical difference when comparing the test to reference product. RIF was bioavailable with respect to Cmax, but when comparing the test to the reference, their was a statistically significant difference. For INH and RIF respectively, the parameters, AUCT and AUCI showed no statistical significantly different results relative to the reference product.

Bioequivalence as presented by Table 18 was satisfactory for parameters AUC and AUCI for the drugs INH and RIF, but not for Cmax which showed bio-inequivalence. The product formulation was declared successful based on bioavailability and bioequivalence data.

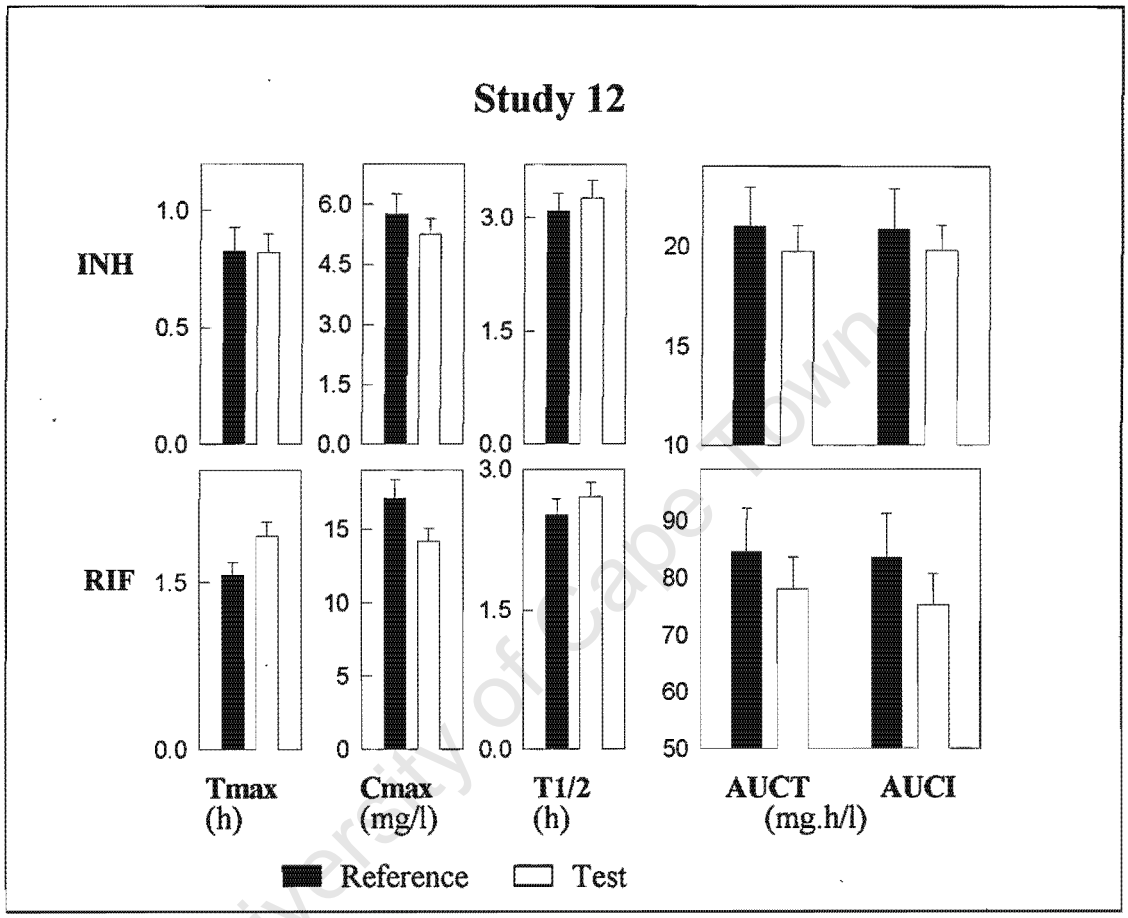


Fig. 14 Bioavailability results of study 12

Table 18. Bioequivalence results of study 12

Study 12 N=22 HV	Median/ Point Estimate	Test/ Reference Ratio	90% Confidence Interval	% Span
Isoniazid (INH)				
Cmax	93.66	1.00	75.24-111.38	36.14
AUC	95.94	1.07	**84.19-109.38**	25.19
AUCI	97.85	1.08	**86.43-111.83**	25.41
Rifampicin (RIF)				
Cmax	84.59	0.87	78.63-91.05	12.42
AUC	92.77	1.00	**88.15-99.49**	11.34
AUCI	90.89	0.99	**87.27-98.33**	11.05

Study 13

The objective of the study was to compare the bioavailability in healthy volunteers of Rifafour E275 fixed combination tablet consisting, of rifampicin , isoniazid, pyrazinamide and ethambutol as a single dose to, the reference tablets of rifampicin, isoniazid, pyrazinamide and ethambutol, as a single dose. The second objective was to establish whether the fixed dose combination formulation Rifafour E275 fixed combination formulation, is bioequivalent to equivalent doses of the drugs in the reference products.

The study concluded (Fig 15), that there was no statistical significant difference in all parameters Cmax, AUCT and AUCI for INH, RIF, PZA and ETB respectively, relative to the reference product.

Bioequivalence results (Table 18), indicated confidence intervals within the required limits for Cmax, AUCT and AUCI for the drugs INH RIF, PZA and

ETB. The product formulation was declared successful based on both bioavailability and bioequivalence data.

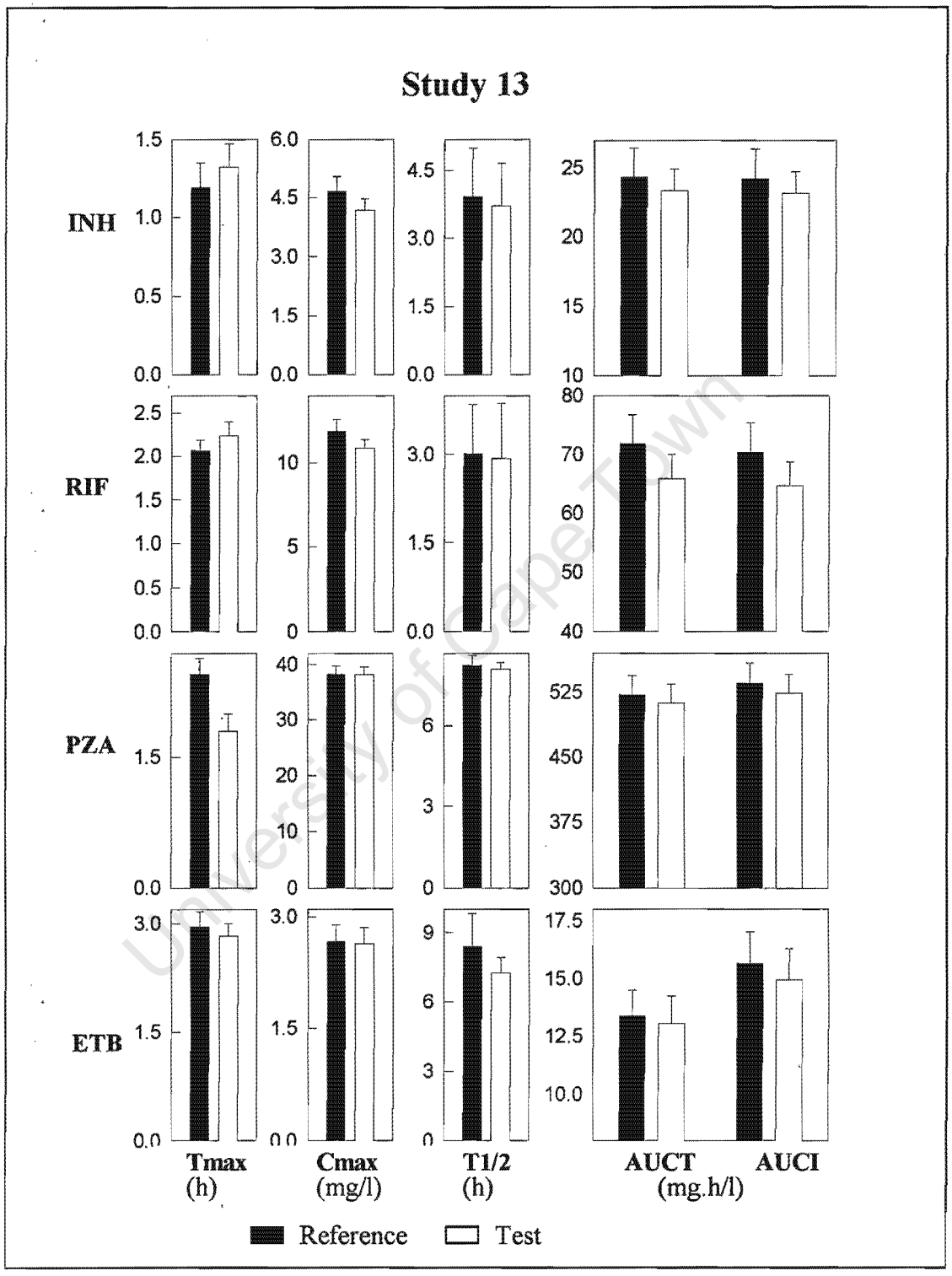


Fig 15. Bioavailability results of study 13

Table 19. Bioequivalence results of study 13

Study 13 N=24 HV	Median/ Point Estimate	Test/ Reference Ratio	90% Confidence Interval	% Span
Isoniazid (INH)				
Cmax	92.80	0.97	**82.3-105.1**	22.8
AUC	100.4	1.06	**91.0-111.2**	20.2
AUCI	101.4	1.07	**91.7-111.7**	20.0
Rifampicin (RIF)				
Cmax	91.5	0.95	**85.5-100.8**	15.3
AUC	92.5	0.95	**87.0-97.8**	10.8
AUCI	92.6	0.94	**87.1-97.4**	10.3
Pyrazinamide (PZA)				
Cmax	100.2	1.02	**96.0-106.0**	10.0
AUC	98.8	1.00	**95.4-103.0**	7.6
AUCI	98.4	1.00	**94.6-102.7**	8.1
Ethambutol (ETB)				
Cmax	100.4	1.15	**84.1-118.5**	34.4
AUC	96.8	1.21	**85.3-113.7**	28.4
AUCI	102.9	1.28	**86.6-120.8**	34.2

3.2 Summary of bioavailability and bioequivalence of studies

Table 20 gives an overall assessment of the 13 studies. The symbols used in the table are defined below and the legend depicts the outcome of the extended time protocol investigation based on the statistical results.

✓= No significant difference for bioavailability data and within 90% confidence interval of 80-125% for bioequivalence analysis. The Student – t- Test (parametric) and Wilcoxon Signed rank Test (non parametric) for bioavailability was similar.

✖= Significant difference for bioavailability data and not within 90% confidence interval of 80-125% for bioequivalence analysis.

✖✓= Dissimilar results for Student –T- Test (parametric) and Wilcoxon Signed rank Test (non parametric) for bioavailability

Legend to define overall study conclusion

	Bioavailability Only- Bioequivalence not within confidence interval
	Bioequivalence Only
	No bioavailability, No bioequivalence
	Bioavailability and Bioequivalence

Table 20. Summary of bioavailability and bioequivalence results

		Bioavailability					Bioequivalence			
Study	Drug	Tmax	Cmax	T1/2	AUC	AUCI	Cmax	AUC	AUCI	
1.1	INH	✓	✓	✓	✓	✓	✓	✓	✓	
	RIF	✓	✓	✓	✓	✓	✗	✗	✗	
1.2	INH	✓	✓	✓	✓	✓	✓	✓	✓	
	RIF	✓	✓	✓	✓	✓	✗	✗	✗	
2	INH	✗	✓	✓	✗	✗	✓	✗	✓	
	RIF	✗	✓	✓	✓	✓	✓	✓	✓	
	PZA	✓	✓	✗	✗	✓	✓	✓	✓	
	ETB	✗✓	✓	✓	✓	✓	✓	✗	✗	
3	INH	✓	✗	✓	✗✓	✗✓	✗	✗	✓	
	RIF	✓	✓	✓	✗	✗	✗	✗	✗	
	PZA	✓	✓	✓	✓	✓	✓	✓	✓	
4	INH	✓	✓	✗	✓	✓	✓	✓	✓	
	RIF	✓	✓	✓	✗	✗✓	✗	✗	✗	
	PZA	✓	✓	✓	✓	✓	✓	✓	✓	
5	INH	✓	✓	✓	✓	✓	✓	✓	✓	
	RIF	✓	✓	✓	✓	✓	✗	✓	✓	
6	RIF	✓	✓	✓	✓	✓	✓	✓	✓	
7	INH	✓	✓	✓	✓	✓	✓	✓	✓	
	RIF	✓	✓	✓	✓	✓	✓	✓	✓	
	PZA	✓	✓	✓	✓	✓	✓	✓	✓	
	ETB	✓	✓	✓	✓	✓	✓	✓	✓	
8	RIF	✓	✓	✓	✓	✓	✓	✓	✓	
	DRIF	✓	✓	✓	✓	✓	✓	✓	✓	
9	INH	✓	✗	✓	✓	✓	✗	✓	✓	
	RIF	✗	✗	✓	✗	✗	✗	✗	✗	
10	INH	✓	✗	✓	✗	✗	✗	✓	✓	
	RIF	✓	✓	✓	✓	✓	✓	✓	✓	
	PZA	✓	✓	✓	✗	✗	✓	✓	✓	
11	INH	✓	✓	✓	✓	✓	✓	✓	✓	
	RIF	✓	✓	✓	✓	✓	✓	✓	✓	
12	INH	✓	✓	✓	✓	✓	✗	✓	✓	
	RIF	✗	✗	✓	✓	✓	✗	✓	✓	
13	INH	✓	✓	✓	✓	✓	✓	✓	✓	
	RIF	✓	✓	✓	✓	✓	✓	✓	✓	
	PZA	✗	✓	✓	✓	✓	✓	✓	✓	
	ETB	✓	✓	✓	✓	✓	✓	✓	✓	

3.3 Conclusion of studies using extended time protocol

Information obtained from bioavailability data from the respective studies presented in Figures 2-15 and Tables 6-19 for bioequivalence together with summary Table 20 were used to determine the outcome of each study investigated.

In conclusion, 13 studies that each consisted of test and reference products were investigated. Eight studies were declared successful based on both bioavailability and bioequivalence results. These studies were, Study 2, 5, 6, 7, 8, 11, 12 and 13. Three studies were declared successful based only on bioavailability results, as the bioequivalence results did not comply with the necessary confidence interval limit of 80-125%, as required. One study, Study 10 was declared successful based only on bioequivalence, as the reference and test products were both bioavailable, but statistically significantly different with respect to INH. Study 9 was declared not a successful product, as the drugs were bioavailable within the required limits, but statistically significantly different when comparing the test to reference products. The bioequivalence results for Study 9 was also outside the desired confidence limit of 80-125% for RIF, rendering the test product formulation unsuccessful.

Chapter 4

Minimum Time Points Protocol Results

4.1 Introduction

In this chapter the results obtained using the time protocol schedule presented in Chapter 2 (Table 5) were used to determine the minimum time point schedule. The extended time (phase 1 analysis) presented in Chapter 3 consisted of 15 time points (0, 0.25, 0.5, 1.0, 1.5, 2, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0, 36.0, 48.0). This is compared to the minimum time point protocol (phase 2) that consists of 11 time points in hours (0, 0.25, 0.5, 1.0, 1.5, 2, 2.5, 3.0, 4.0, 6.0, 8.0) and the phase 3 minimum time protocol that consists of 6 time points (0, 1.0, 2.0, 4.0, 6.0, 8.0).

The data is presented in graphic form to provide a holistic view and ease of interpretation and understanding of the data. Bioavailability and bioequivalence data obtained in Chapter 3 as well as the derived conclusion served as a finger print to determine the optimum minimum time protocol to derive similar conclusions. For the purposes of the investigation in this chapter, only Cmax, AUCT, AUCI were considered for both bioavailability and bioequivalence analysis.

4.2 Graphic depiction of phase 1, 2 and 3 time protocol

Fig 18 (Study 2) is used as an example to give a descriptive layout and template of the graphic information of all the studies investigated. Each drug investigated within a given study comprises three panels from left to right reflecting Cmax (mg/l), AUCT(mg.h/l) and AUCI(mg.h/l) for both bioavailability and bioequivalence. The left and centre panels deal with bioavailability information Cmax(mg/l) and the centre panel with AUCT(mg.h/l) and AUCI(mg.h/l) information.

For bioequivalence the base of the panel is defined as 80% and the upper limit of the confidence interval 125%.

The bar graphs with standard error of the mean indications are represented by a dark reference bar and clear test bar. Each set of bar graphs represents phase 1, 2 and 3 time protocols from left to right within each panel respectively.

The bioequivalence information follows the same sequence. For acceptable bioequivalence the bar has to fall within the limits of the base of the panel and the upper limit of 125%. Each bar represented has an upper and lower limit that denotes the percentage span. The mid point of each bar is defined as the point estimate value.

Study 1

In summary phase 2 and phase 3 analysis of Study 1.1 (Rifinah 150) and Study 1.2 (Rifinah 300) that investigated two different dosages relative to a reference, yielded the following results: both Study 1.1 and Study 1.2 (figure 16 and 17), demonstrated comparable bioavailability for all parameters investigated being Cmax, AUCT and AUCI with no significant difference to the reference. The phase 2 analysis represents the desired minimum time points as the extended time points in Study 1.1 and Study 1.2 in phase 1 went up to a maximum of 12 hours.

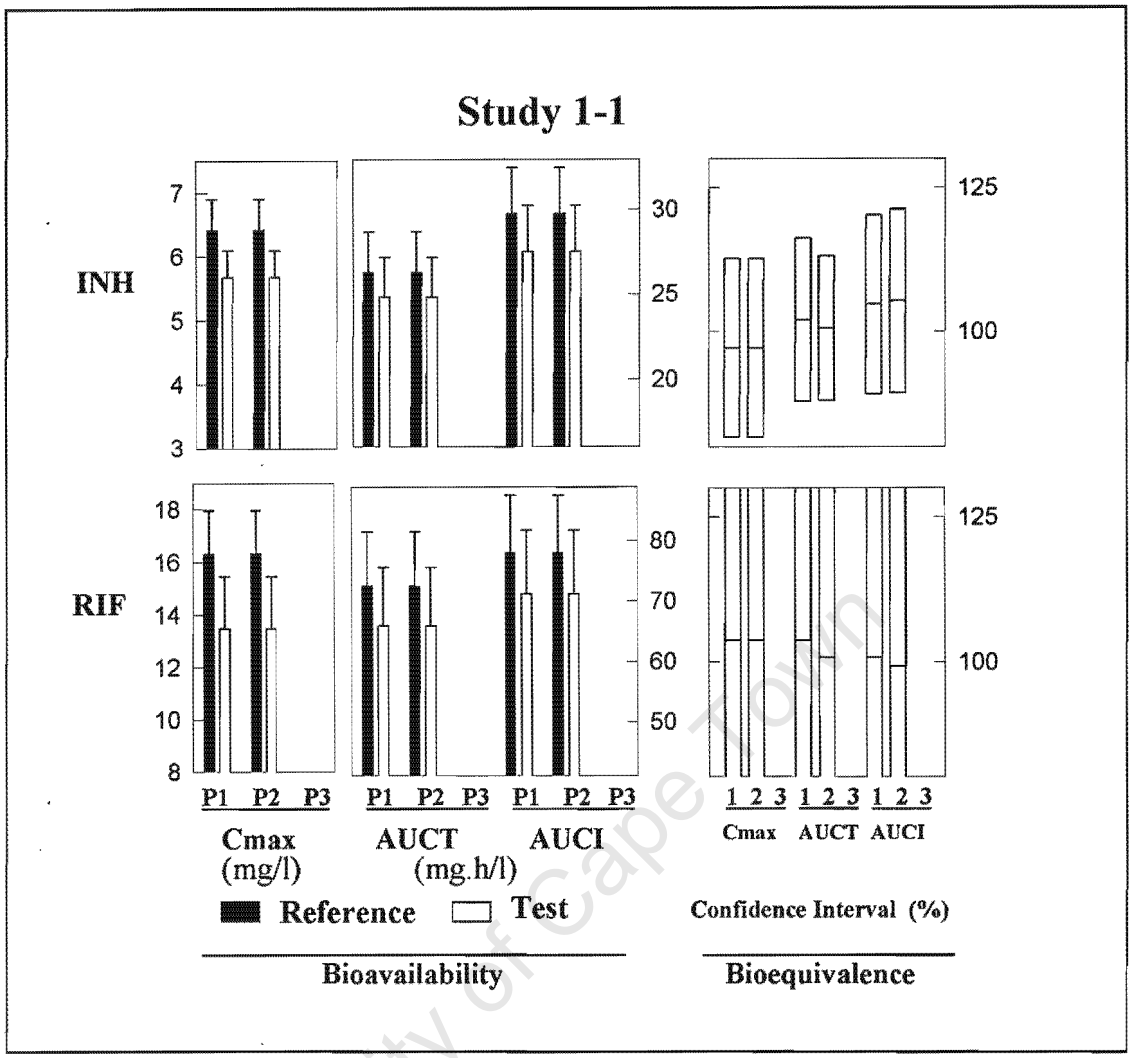


Fig 16. Bioavailability and bioequivalence results all phases study 1.1

The two assumptions for the bioequivalence analysis in phase 1 (Chapter 3) were applied: (1) that subjects received the reference drug in period one and the test drug in period two, and that, (2) for purposes of the analysis, the first eight subjects received the drug in the order reference then test and the second eight received it in the reverse order. The bioequivalence analysis for Study 1.1 and 1.2 in phase 2 was within limits for the INH parameters of Cmax , AUCT and AUCI . For RIF, bio-inequivalence was shown for all the parameters in phase 2 for Study 1.1 and 1.2, respectively.

The product formulation was successful based on comparable bioavailability only, yielding similar results in both phases and consistently complied with the bioequivalence limits for INH. With respect to Study 1.1 and 1.2 (Fig 16 and

17), RIF parameters Cmax, AUCT and AUCI, consistently did not fall within the limits required for bioequivalence to be acceptable across the various phases investigated.

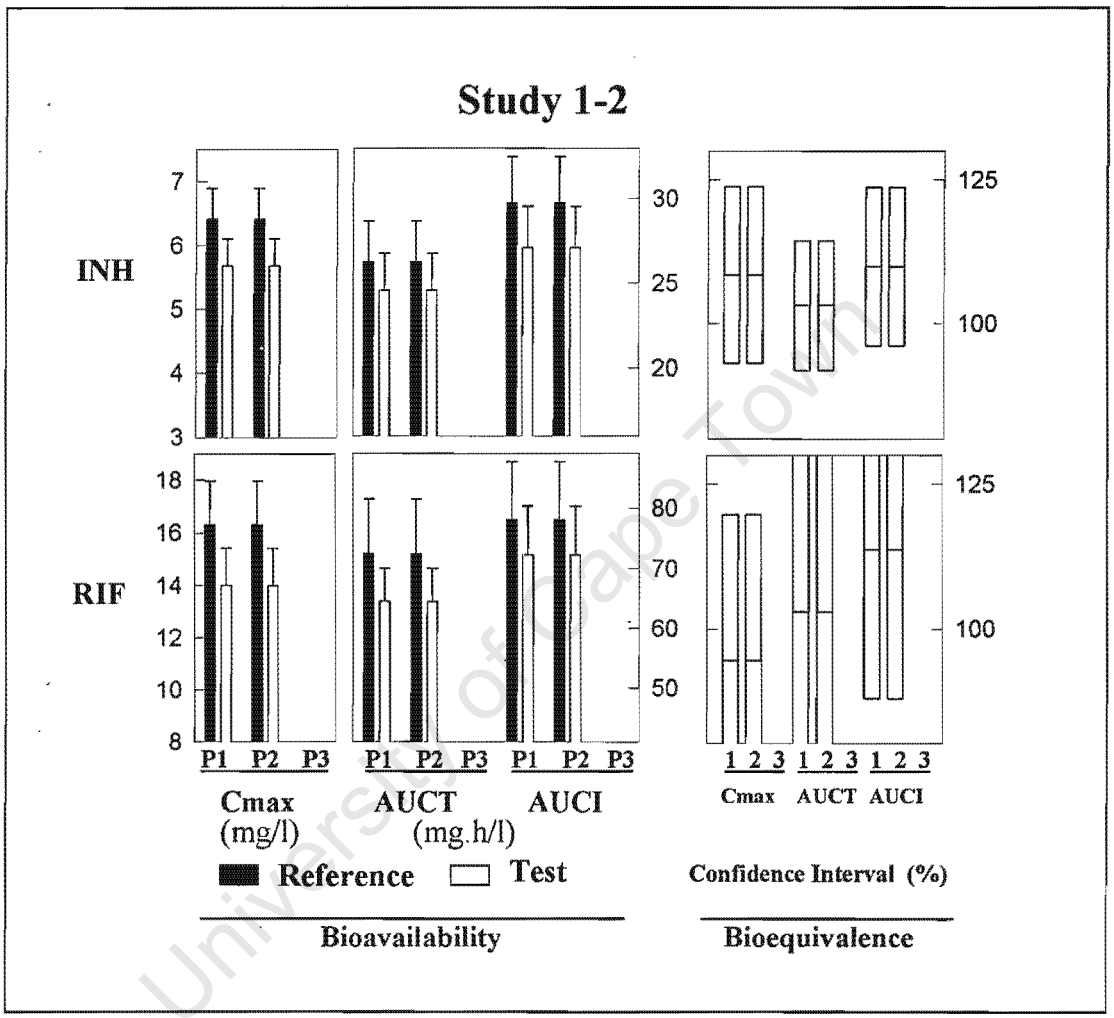


Fig 17 Bioavailability and bioequivalence results all phases study 1.2

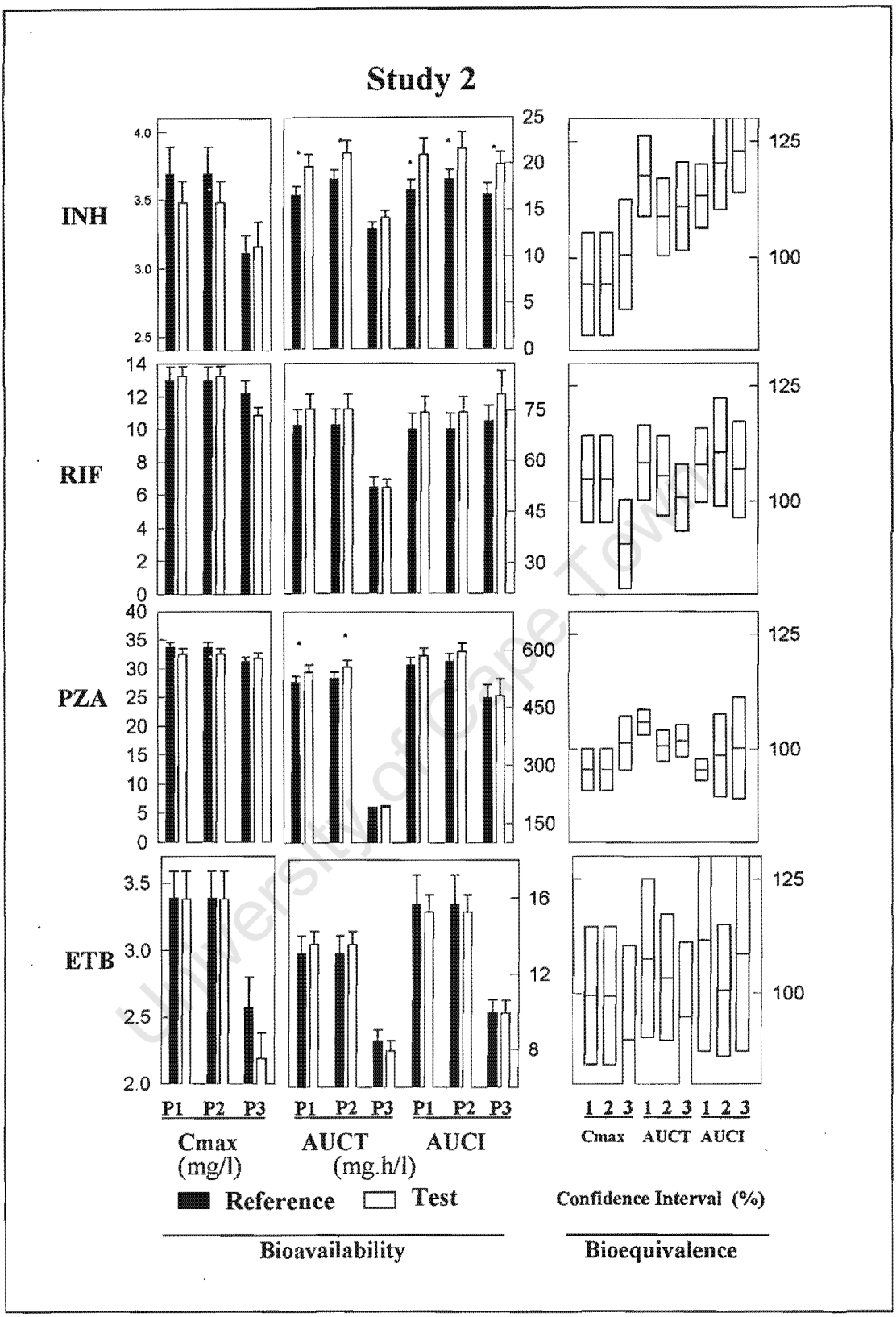


Fig 18 Bioavailability and bioequivalence results all phases study 2

Study 2

In summary the phase 2 and phase 3 analysis of Study 2 (Myrin-P) (figure 18) demonstrated comparable bioavailability for Cmax, for all 4 drugs with no significant difference to the reference and to that of phase 1 analysis. The results for INH parameters, AUCT and AUCI, were significantly different across phases and reflect the extended time point results. Bioavailability for AUCT and AUCI results for RIF and ETB consistently showed no significant difference across phases although the apparent AUCT values are lower in phase 3. The AUCI values remained consistent for RIF across phases, whereas those for ETB dropped relatively, but were not significantly different to the reference in phase 3. PZA showed no significant difference for the AUCI parameter, across the phases investigated. AUCT for PZA showed consistent significant difference from phase 1 to phase 2, but no significant difference in phase 3 using the desired minimum time points. The apparent PZA AUCT value is also lower to that of phase 1 and phase 2 as to be expected, due to the selected time-concentration profile.

The bioequivalence analysis was within limits for Cmax of INH across the different phases based on the established criteria. For AUCT, INH was marginally out of the confidence limits for phase 1. In phase 2 and 3 the values were within confidence limits. For AUCI INH the phase 1 bioequivalence values were within limits, but not in phase 2 and 3. For RIF and PZA all parameters (Cmax, AUCT and AUCI) were within the confidence limit for bioequivalence. For ETB phase 1, only Cmax was within limits with the AUCT value being marginally out on the upper end of the confidence interval. Bio-inequivalence was shown based on AUCI. In phase 2 ETB showed bioequivalence for all parameters. The phase 3 results yielded bio-inequivalence based on Cmax. The AUCT value were marginally out at the bottom end of the interval and the AUCI to the upper end of the interval for ETB.

Phase 1 results yielded the test product formulation successful relative to the reference product, based on bioavailability and bioequivalence. Phase 2 and phase 3 had comparable results for bioavailability. Similar results across phases were also registered to determine bioequivalence for INH, RIF and PZA with the exception of ETB in phase 3 that showed bio-inequivalence. The ETB AUCT value was however marginally out at the lower end of the interval.

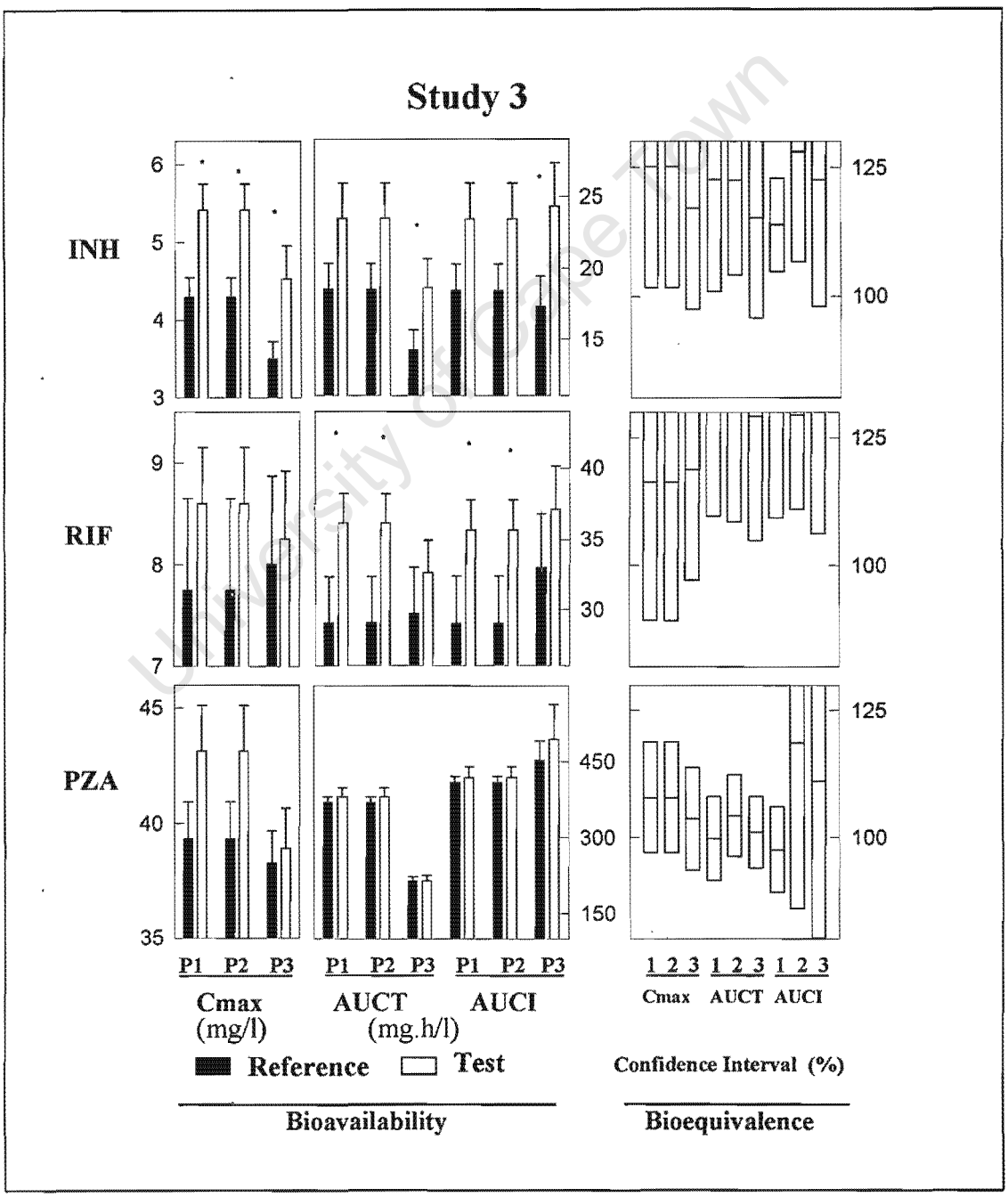


Fig 19. Bioavailability and bioequivalence results all phases study 3

Study 3

In summary (Fig 19), the phase 2 and phase 3 analysis of Study 3 (Rifcin Pedz) demonstrated adequate consistent bioavailability on all three drugs. The test formulation showed slightly higher values for Cmax relative to the reference throughout, with the exception of INH that was higher but also showed statistically significantly difference to that of the reference. This was apparent across all phases.

For INH, AUCT and AUCI were also consistent across phases showing a significant difference to that of the reference based on the parametric test but not for the non-parametric test, which gave p-values of 0.07 and 0.06 for AUCT and AUCI, respectively, for phase 1 and phase 2, but a significant difference for both AUCT and AUCI in phase 3 where the p-values were less than 0.05.

Rifampicin showed a significant difference for both AUCT and AUCI in phase 2 but not in phase 3, while pyrazinamide showed complete and consistent bioavailability across phase 2 and phase 3.

Bioequivalence was shown completely for PZA, for the parameters Cmax and AUCT for phase 2 and 3, but not for AUCI. RIF showed consistent bio-inequivalence across phases and across parameters in phase 2 and 3, noting that AUCI showed bioequivalence in phase 1. The conclusion with respect to phase one remains consistent in that the product was successful as bioavailability was within limits with respect to Cmax. However the bioequivalence was unsuccessful with respect to INH and RIF, PZA being the exception.

Study 4

In summary (Fig 20) the phase 2 and phase 3 analysis of Study 4 (Rifcin Co) demonstrated comparable bioavailability on all three drugs with reference to Cmax that was not significantly different to that of the reference with the exception of RIF in phase 3 that showed a significant difference.

With respect to AUCT and AUCI both INH and PZA showed no significant difference across the phases. There was a drop in AUCT values in phase 3 of both INH and PZA, but the AUCI values remained consistent and similar across the phases. For RIF, the AUCT showed a significant difference in phase 1 and phase 2, but not in phase 3 with the drop in AUCT values when the minimum time points were utilised. AUCI values for RIF remained consistent across phases with no significant difference.

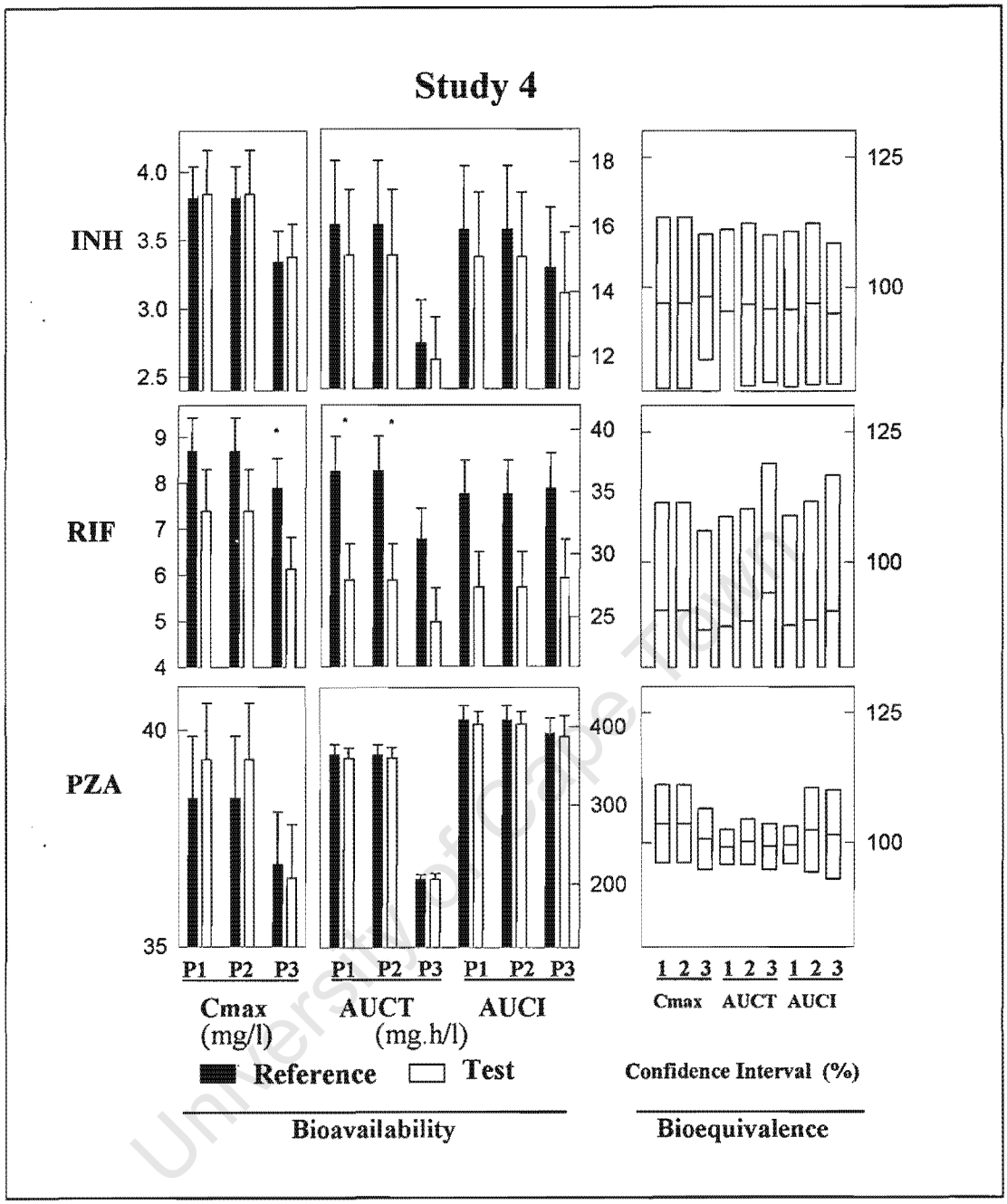


Fig 20. Bioavailability and bioequivalence results all phases study 4

Bioequivalence was within the 90% confidence interval for Cmax, AUCT, AUCI for INH and PZA across the phases investigated. RIF showed bio-inequivalence consistently across all phases and all parameters.

The conclusion summary is consistent, showing adequate comparable bioavailability for the formulation with reference to Cmax, making the formulation successful. However based on the bio-inequivalence of RIF, where

the confidence interval fell outside the lower confidence interval as required, the test product was found to be unsuccessful.

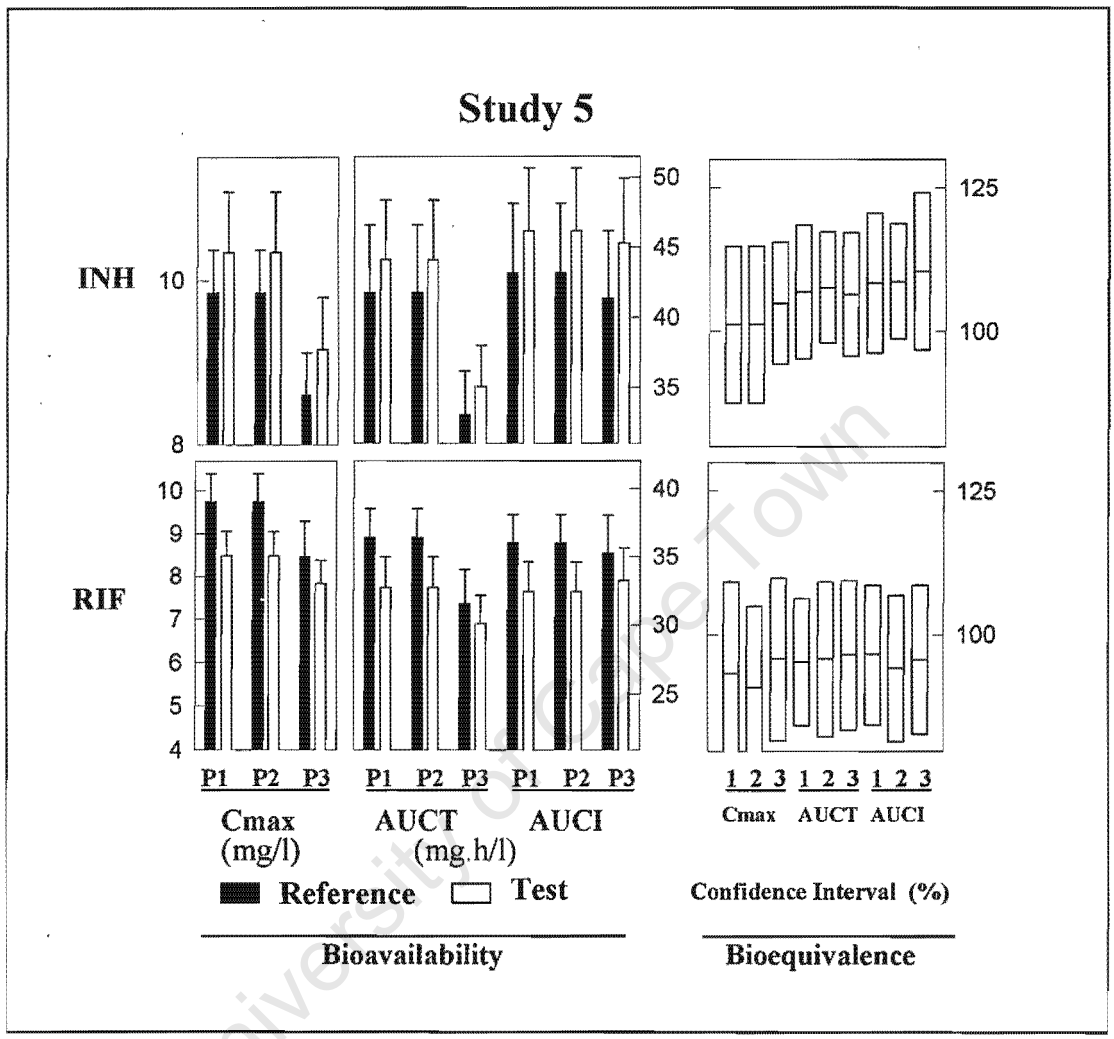


Fig 21. Bioavailability and bioequivalence results all phases study 5

Study 5

In summary (Fig 21) the phase 2 and phase 3 analysis of Study 5 (Rifcin Ped) demonstrated bioavailability for both INH and RIF for Cmax, AUCT and AUCI. Bioequivalence was shown for INH consistently for all phases and all parameters. Bioequivalence was also shown for RIF, for the AUCT and AUCI parameters across phases 1 to 3. RIF Cmax showed bio-inequivalence for phase 1 and 2 being marginally out at the lower confidence interval, but not in phase 3 where bioequivalence was registered.

The conclusion summary is thus consistent with that of the extended time protocol showing successful product formulation based on bioavailability and bioequivalence.

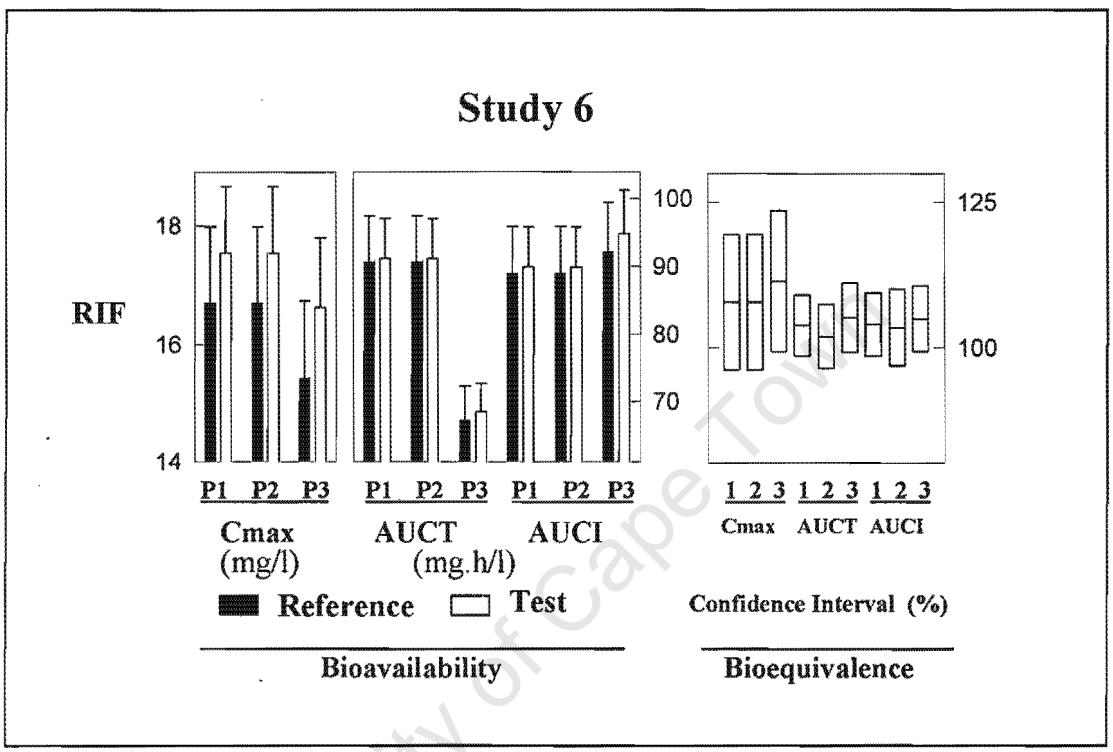


Fig 22. Bioavailability and bioequivalence results all phases study 6

Study 6

In summary (Fig 22) the phase 2 and phase 3 analysis of Study 6 (Rifadin) demonstrated consistently similar bioavailability for RIF to that of the reference formulation of Rimactane across phases 2 and 3. The Cmax of the test was marginally higher than that of the reference but not significantly different to it. The Cmax obtained using the minimum time points protocol in phase 3 presents a lower but not significantly different result for both test and reference. This also presents a lower AUCT value in phase 3 but not a significantly different result. AUCI remained consistently similar across all phases. Bioequivalence was shown for Cmax, AUC and AUCI across all phases. The conclusion summary remained consistent in that the product formulation was successful based on bioavailability and bioequivalence.

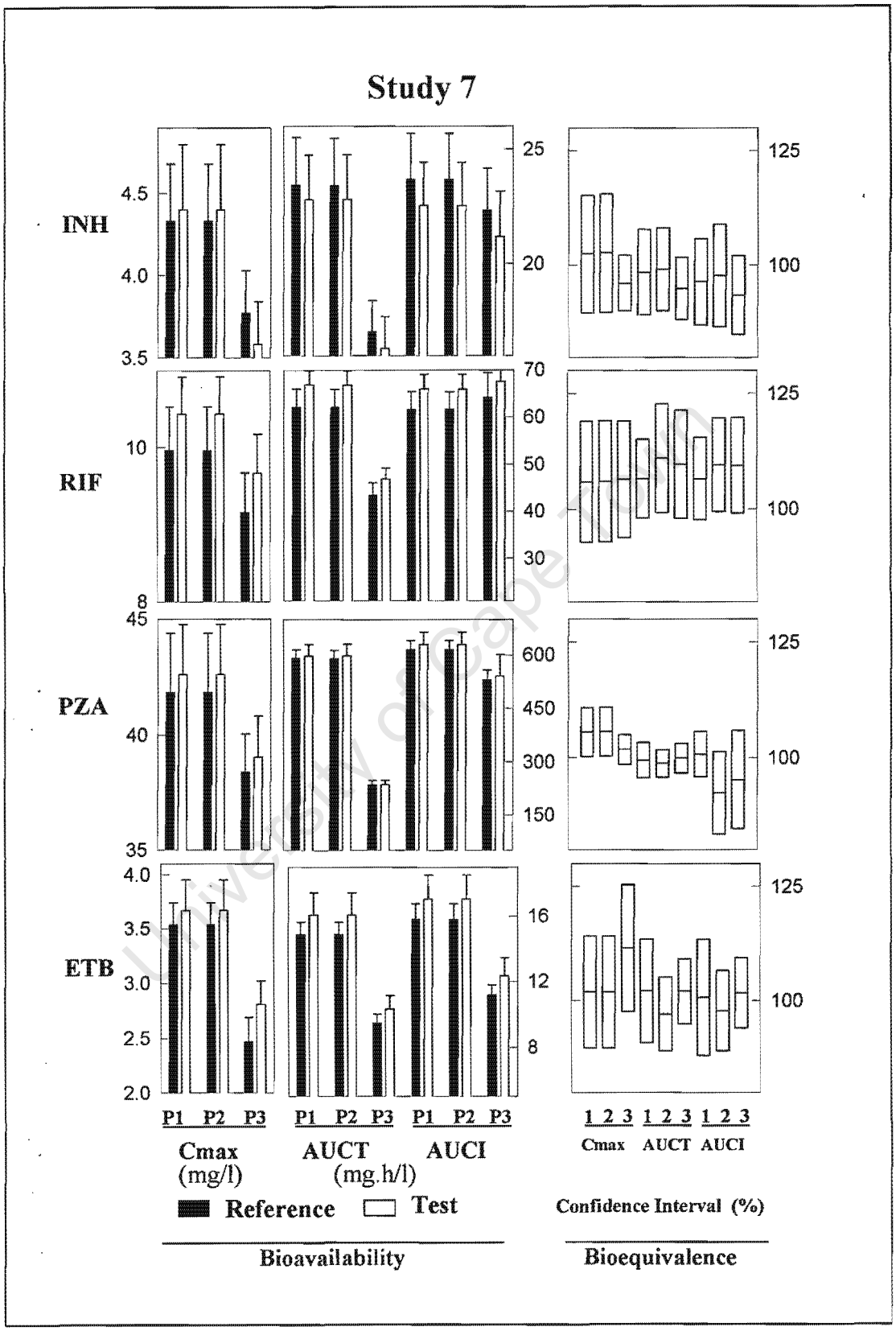


Fig 23. Bioavailability and bioequivalence results all phases study 7

Study 7

In summary (Fig 23) the phase 2 and phase 3 analysis of Study 7 (Rifafour) demonstrated bioavailability consistently across all phases with no significant difference to the reference for all four drugs, for the parameters measured.

Bioequivalence was satisfactorily complied with for all parameters of Cmax, AUCT and AUCI and for all the drugs INH, RIF, PZA, ETB with the exception of Cmax for ETB in phase 3 that was marginally out on the upper end of the limit. The summary conclusion across phases remains consistent in that the product formulation was successful based on both bioavailability and bioequivalence.

Study 8

In summary (Fig 24), the phase 2 and phase 3 analysis of Study 8 (WHO) assayed for only RIF and DRIF but consisting of a three drug combination INH, RIF and PZA, demonstrated bioavailability for the drug RIF and metabolite DRIF under investigation for parameters Cmax, AUCT and AUCI across all phases. The mean average for Cmax for the test product was marginally higher and similar than the reference although not significantly different. The apparent AUCT was also lower in phase 3 due to minimum time points used but not significantly different for both the RIF and metabolite DRIF. The AUCI values were also consistent and not significantly different.

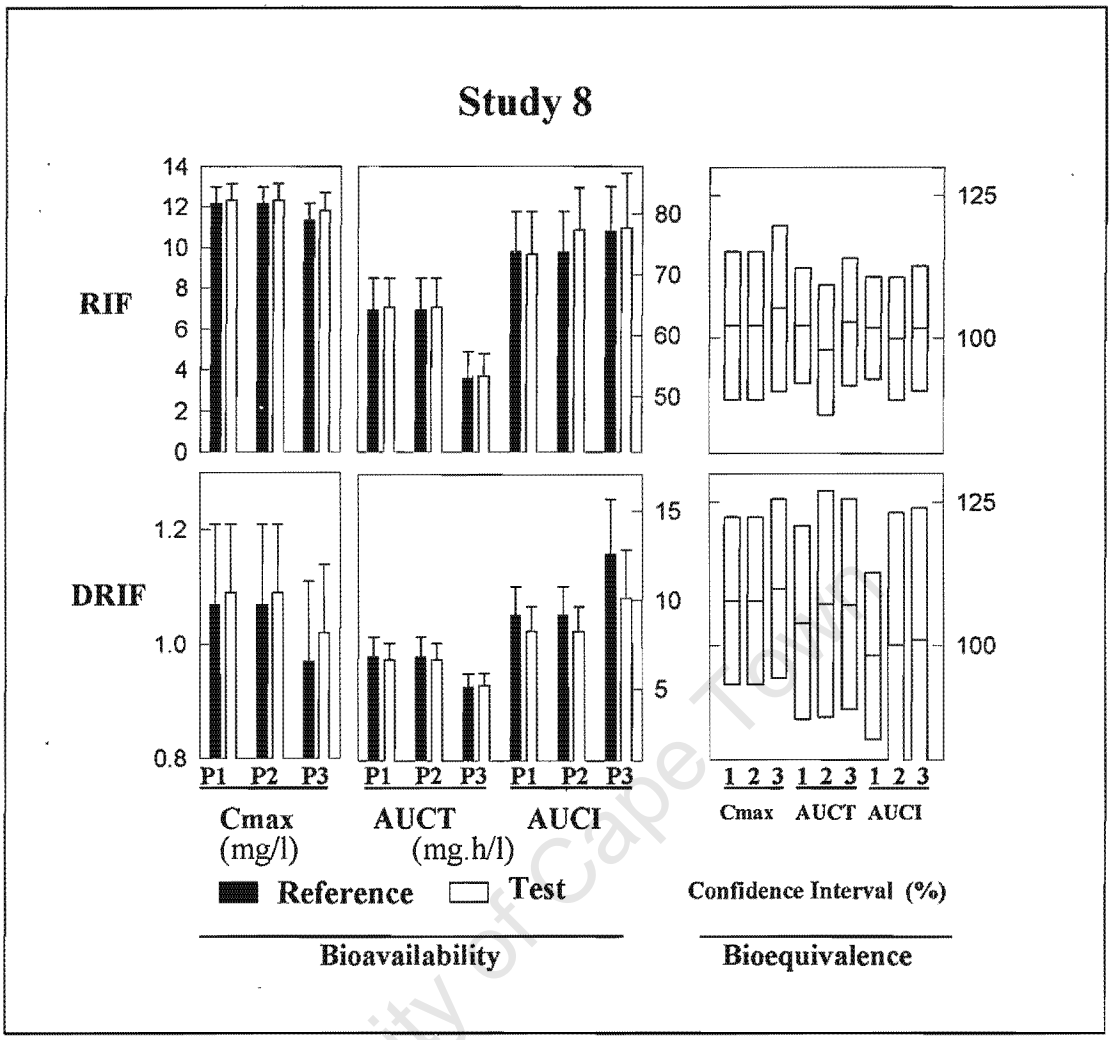


Fig 24. Bioavailability and bioequivalence results all phases study 8

Bioequivalence analysis was satisfactory for RIF for Cmax, AUCT, AUCI. The metabolite DRIF was within limits for Cmax phase 1 and phase 2, but marginally out on the upper limit for AUCT and on the lower limit for AUCI. For phase 3, Cmax and AUCT were marginally out on the upper limit and AUCI on the lower limit. The conclusion summary is thus consistent with the parent drug RIF being successful across all phases for this product formulation based on bioavailability and bioequivalence. Bioequivalence analysis was also adequate for the metabolite across all phases, although less convincingly than RIF.

Study 9

In summary (Fig 25) the phase 2 and phase 3 analysis of Study 9 (Rifinah Ped) demonstrated consistent bioavailability for INH and RIF across all phases with INH's AUCT and AUCI not being significantly different and that of Cmax showing a consistent significantly different result. For RIF Cmax , AUCT and AUCI showed a consistently significant result across phases.

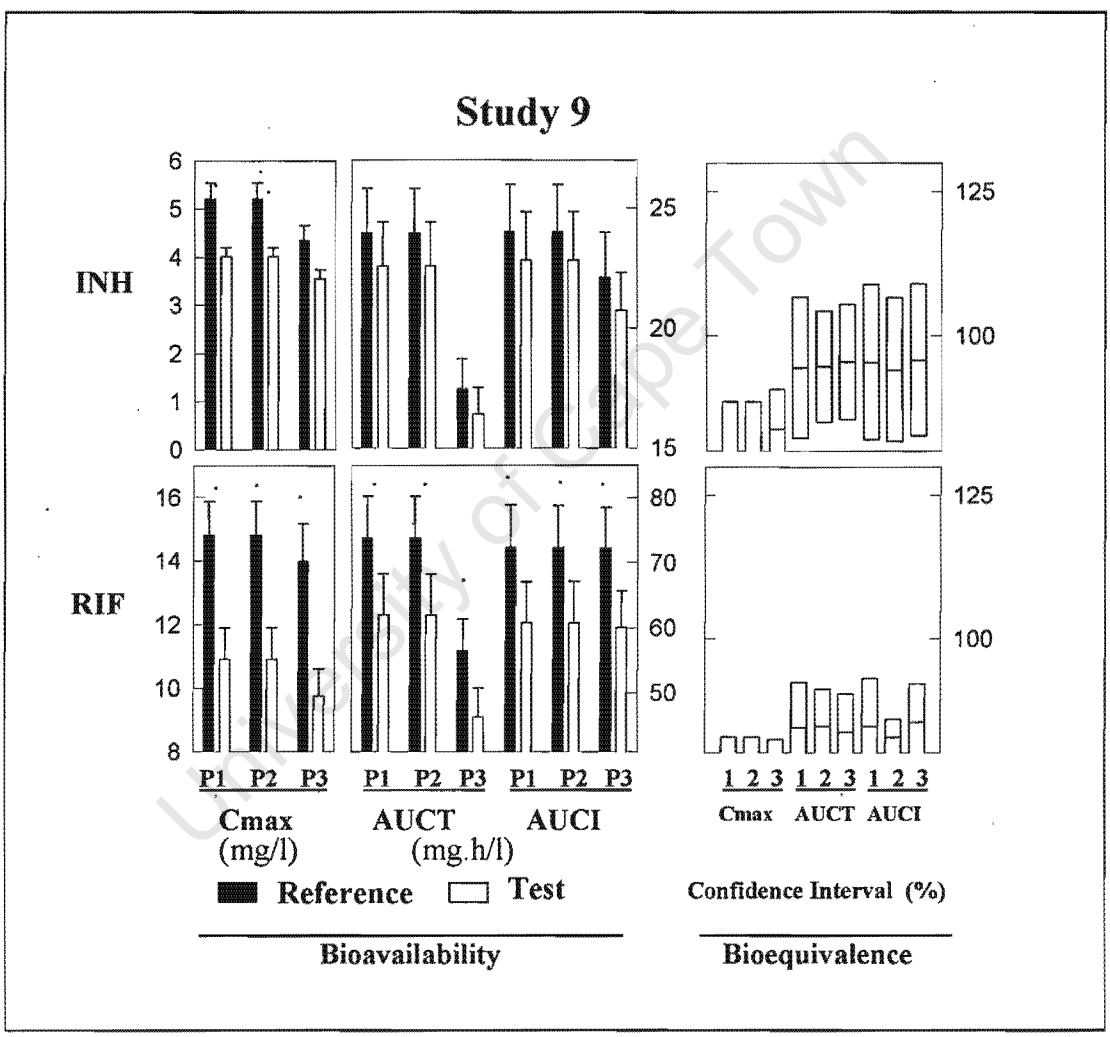


Fig 25. Bioavailability and bioequivalence results all phases study 9

Bioequivalence analysis was satisfactory for INH in AUC and AUCI but not for Cmax across the three phases. All parameters for RIF showed bio-inequivalence across all phases.

The conclusion summary with the extended time protocol to that of phase 2 and phase 3 remains consistent in that the product formulation as a whole was successful based on bioavailability only and not on bioequivalence due to RIF.

Study 10

In summary (Fig 26) the phase 2 and phase 3 analysis of Study 10 (Rifater Ped) demonstrated consistent bioavailability for both RIF and PZA with no significant difference in the average mean relative to the reference in Cmax across all phases. Cmax for INH showed consistently similar results across all phases as well as showing a significant difference between test and reference across all phases. AUCT and AUCI for RIF were similar across all phases with only AUCT being lower due to the minimum time points schedule in phase 3. No significant difference was observed for bioavailability for AUCT and AUCI for RIF but for both INH and PZA these parameters was consistently significantly different across all phases, but similar.

The bioequivalence analysis was satisfactory and consistent within the limits for all parameters for RIF and PZA across all phases. Bioequivalence was shown for AUC and AUCI with bio-inequivalence for Cmax across all phases for INH. The summary conclusion for the extended time points remains consistent with product formulation being successful based on bioavailability and bioequivalence.

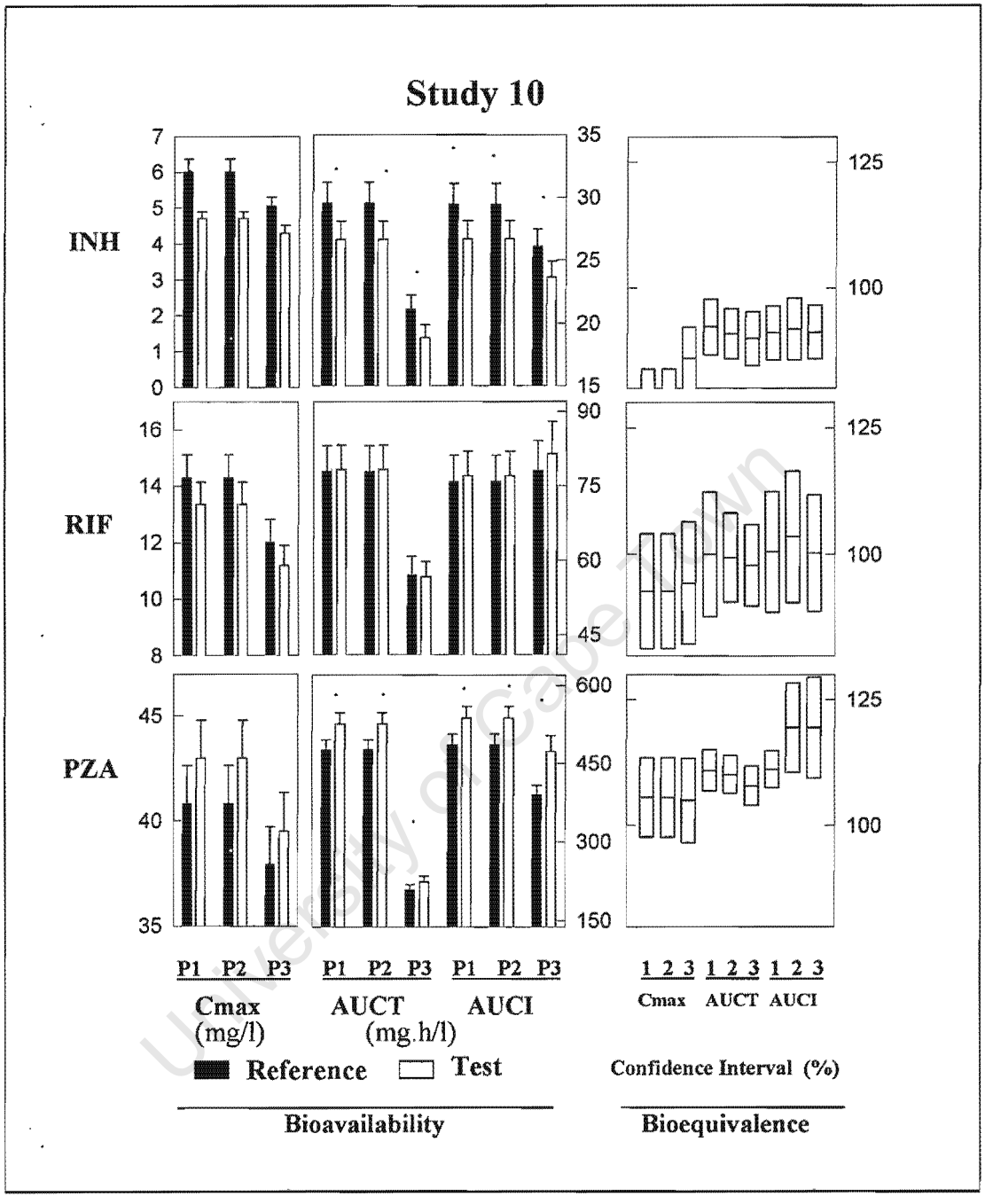


Fig 26. Bioavailability and bioequivalence results all phases study 10

Study 11

In summary (Fig 27) the phase 2 and phase 3 analysis of Study 11 (Rifinah 150/150) demonstrated consistent bioavailability and was similar for all parameters for both drugs INH and RIF across all phases.

Bioequivalence was also consistently and adequately achieved within the confidence interval for both INH and RIF based on Cmax, AUCT and AUCI. The summary conclusion is therefore consistent for the product formulation making it successful for both bioavailability and bioequivalence.

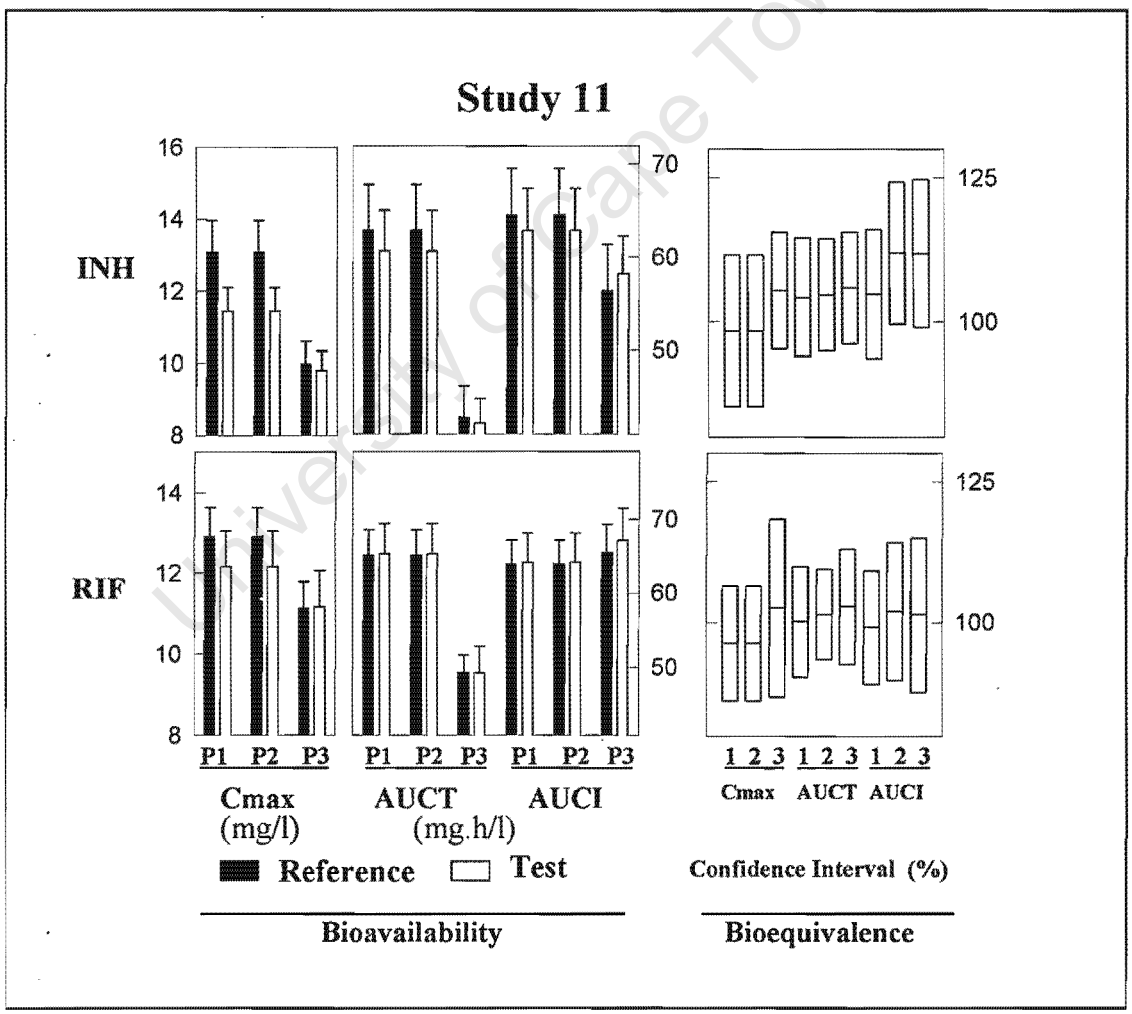


Fig 27. Bioavailability and bioequivalence results all phases study 11

Study 12

In summary (Fig 28) the phase 2 and phase 3 analysis of Study 12 (Rifinah 150/75) demonstrated consistent bioavailability for INH across all phases that was not significantly different for Cmax relative to the reference product. RIF Cmax was also consistently significantly different across all phases.

For AUCT and AUCI both RIF and INH were consistent across all phases and not significantly different across all phases with the exception of RIF AUCT that showed a significant difference in phase 3.

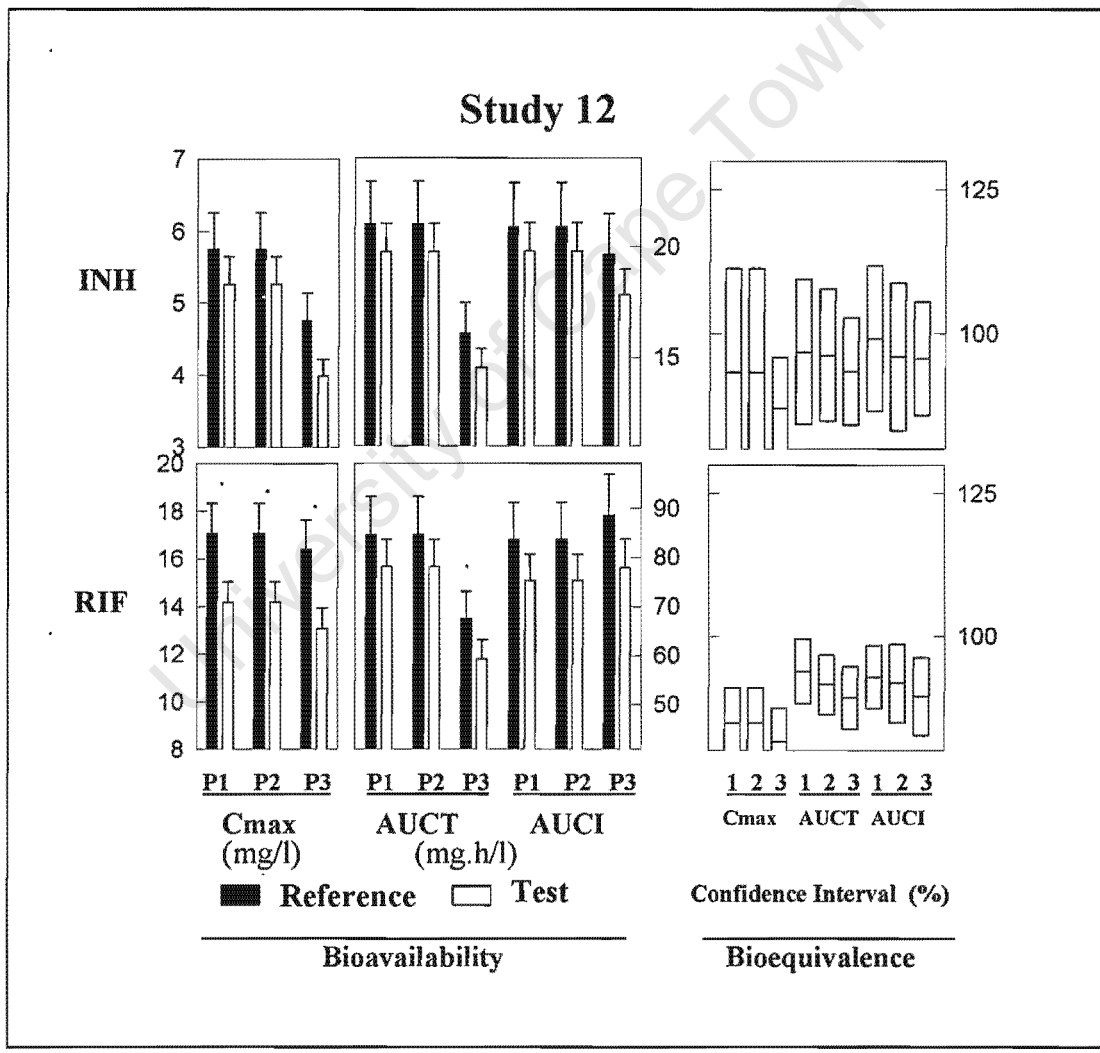


Fig 28. Bioavailability and bioequivalence results all phases study 12

Bioequivalence was consistently satisfactory for parameters AUC and AUCI for both drugs across all phases, but not for Cmax that showed bio-inequivalence

across all phases for both drugs. The conclusion summary for the product formulation was successful and consistent for all phases based on both bioavailability and bioequivalence.

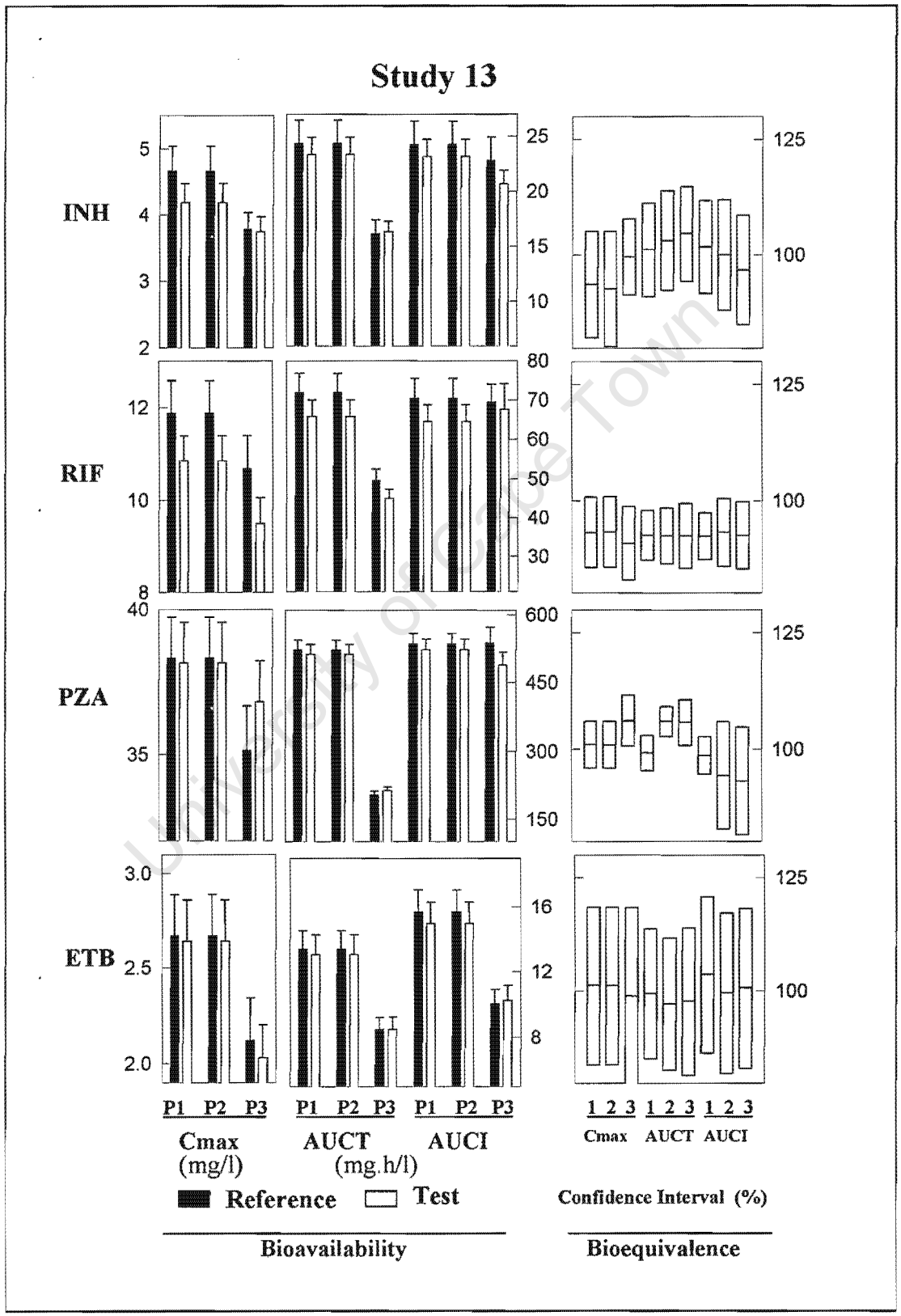


Fig 29. Bioavailability and bioequivalence results all phases study 13

Study 13

In summary (Fig 29) the phase 2 and phase 3 analysis of Study 13 (Rifafour E275) demonstrated consistent bioavailability for all four drugs investigated and for all parameters, showing no significant difference to that of the reference across all phases. The apparent AUCT values for all drugs were also lower in phase 3 due to the minimum time points schedule.

The bioequivalence analysis was also within the limits for all the parameters of Cmax, AUCT and AUCI across all phases, for all the drugs investigated with the exception of Cmax for ETB that was marginally out on the lower limit of the bioequivalence confidence interval in phase 3. The conclusion summary across all phases is thus consistent making the product formulation successful in terms of both bioavailability and bioequivalence.

4.3 Discussion and Conclusion

With respect to the studies investigated for all the drugs within a given study and across all the phases it is apparent that bioequivalence analysis is a sensitive simple tool to assess the overall quality of the studies and to determine the minimum time points needed for such investigations. For a given formulation, the pharmacokinetic parameters of Cmax, AUCT and AUCI, across the phases investigated, showed consistency in the outcome of the results.

For bioavailability, data was generated for Tmax, Cmax, half-life, AUCT and AUCI across all phases and for all drugs but only the parameters of importance, Cmax, AUCT and AUCI were presented in the graphic form.

What is important and has been achieved in this study, is that the Cmax drug concentration values in all 3 phases should fall within range (therapeutic range) as set out in the literature and presented in Table 4. The second point that has

been achieved is when comparing extended to minimum time points consistency in evaluation across phases is maintained to ensure similar conclusions can be derived, with respect to the quality of the product investigated. This process has ensured that the optimum minimum time points were derived. Based on these observations it is evident that the extended time protocol does not provide inherently a greater quality of data than that of minimum time points schedule.

The templates presented in Chapter 3 (Table 6-19) for the extended time protocol that included all pharmacokinetic parameters, remained remarkably similar across all parameters and across all phases in general. In particular, a high level of consistency was found when comparing phase 1 and phase 2 time protocols for all pharmacokinetic parameters, indicating the acceptability of the phase 2 minimum time points protocol.

Observation from the graphical data of the respective studies showed that AUCI and, more especially AUCT, yielded the best results consistently. It is clear that in general AUCT data remained the same for phase 1 and phase 2 and that phase 3 bar charts as expected were lower in magnitude, relatively, due to the only 6 time points being used.

For AUCI data in general and with respect to phase 1, 2 and 3 analysis, bar graph magnitude as presented by the second panel in figures in this chapter, remain constant in particular where drugs RIF and PZA are present in the fixed-dose drug combination. This is however not the case where INH and ETB are present in the combination as the observed magnitude of the graph is constant for phase 1 and 2, but not for phase 3, where the general drop in the magnitude of these bar graphs is observed. In the study where DRIF was measured there was also an increased shift in the observed magnitude of the bar chart in phase 3.

The phase 2 time protocol in general thus provides the optimum time points to yield similar conclusions to those of the extended time protocol with respect to Cmax, AUCT and AUCI for both bioavailability and bioequivalence assessment. The benefit of this finding is that the protocol can contribute towards establishing a policy that is cost effective in the reduced number of samples, the reduced number of analyses, and the potential quicker turn around time to analysis and statistical assessment. Based on current regulatory authority requirements, the phase 2 protocol would be satisfactory in meeting the objectives required for fixed-dose drug combination bioequivalence testing and is comparative to the extended time protocol that is currently in use. This would hopefully also serve as encouragement for the pharmaceutical industry to manufacture fixed-dose drug combinations.

The phase 3 time protocol, from general observations seemed to be acceptable for many of the studies but was not consistent with results obtained in phase 1 and phase 2 protocols in some of the product formulations investigated, in particular in the case of the Cmax and AUCI results. This impacted the bioequivalence assessment in phase 3 due to the highly restricted time points utilised. The benefits of the phase 3 protocol could instead be utilised for ongoing quality assurance after a particular fixed-dose drug combination has been registered by the regulatory authority. Further possibilities that could be explored are establishing whether Cmax/AUC and/or Cmax/AUCI ratios could potentially yield a more reliable level of consistency with the phase 3 time points schedule in mind.

In conclusion, the studies investigated covered different variables that included, fixed-dose drug combinations and formulations from different manufactures. The formulations consisted of tablets and paediatric suspensions. The subject numbers ranged from 16 to 24 subjects in each of respective studies and also different sex. Studies were done in healthy volunteers as well as patients. One study (Fig 24) (Study 8) evaluated both parent compound and metabolite.

Taking the pointers from the discussion and conclusion of this chapter and also the various variables into account, it is apparent that the methodology that was applied to determine the optimum minimum time points was acceptable. The bioavailability parameters of importance, Cmax, AUCT and AUCI were acceptable for the intended investigation to show comparable bioavailability of the test relative to the respective reference formulation in all three phases. Further, the pharmacokinetic parameters, as stated, could also be used successfully to determine if similar quality of the fixed-dose drug formulation was achieved relative to the reference product, utilizing the phase 2, but not phase 3 minimum time point protocol schedule.

Chapter 5
Correlation, Sub-Group and Reduced Subject Analysis

5.1 Correlation analysis

Spearman–r-correlation analysis was done using all data acquired over the 13 studies for the drugs INH, RIF, PZA and ETB. The data was used regardless whether the drug was administered as a FDC or individual formulation. As described in Chapter 4 and based on the observations and conclusions made, the protocol with optimum minimum time points was that of phase 2 (0, 0.25, 0.5, 1.0, 1.5, 2, 2.5, 3.0, 4.0, 6.0, 8.0). The aim of the correlation analysis was to provide added support and thereby verify phase 2 as the optimum minimum time points schedule. The correlation was restricted to phase 1 and phase 2 pharmacokinetic parameters.

Table 21 describes the comparison of the respective pharmacokinetic results presented in all the studies and reinforces the findings in Chapter 4.

Table 21. Correlation Analysis Table of phase1 and phase 2 parameters independently.

	Phase 1			Phase 2		
	Cmax vs AUCT	Cmax vs AUCI	AUCT vs AUCI	Cmax vs AUCT	Cmax vs AUCI	AUCT Vs AUCI
INH	0.87	0.87	1.00	0.90	0.86	0.98
RIF	0.81	0.79	0.98	0.90	0.79	0.91
PZA	0.95	0.94	1.00	0.96	0.94	0.96
ETB	0.99	0.99	1.00	0.99	0.99	1.00

The analysis shows that overall there is good agreement between the Cmax, AUCT and AUCI correlation values. This implies that data obtained with a lesser time point schedule with 11 time points (phase 2), compares well with results obtained using the extended 15 time point schedule (phase 1).

The best correlation is however defined by AUCT vs AUCI in both phase 1 and phase 2 confirming the statistical package to determine similar outcomes with fewer time points. The observation, in increasing order of improvement of correlation in both phases and across all drugs investigated is, Cmax vs AUCT, Cmax vs AUCI and AUCT vs AUCI. It is also apparent that by optimising the time protocol, in this case phase 2, there is an inherent improvement in the quality of the data as shown by the Spearman correlation coefficient (Table 21). The correlation with specific reference to Cmax vs AUCT in both phases and for drugs INH, RIF, PZA and ETB is improved from 0.87, 0.81, 0.95 and 0.99, to 0.90, 0.90, 0.96 and 0.99, respectively.

If it is considered that phase 1 time protocol is presently considered as the "gold" standard, then it becomes appropriate to compare phase 2 as the recommended optimum time protocol to that of phase 1, thereby giving additional justification for phase 2 time protocol's acceptability with respect to Cmax, AUCT and AUCI. Table 22 illustrates, by means of the Spearman–r-correlation, the relationship between phase 1 and phase 2 with respect to the given pharmacokinetic parameters.

Table 22. Correlation Analysis comparison of phase1 and phase2 parameters

		Phase 2		
	Phase1	**Cmax**	**AUCT**	**AUCI**
INH	**Cmax**	1.00	0.90	0.86
	AUCT	0.87	0.99	0.99
	AUCI	0.87	0.98	0.99
RIF	**Cmax**	0.98	0.88	0.77
	AUCT	0.82	0.96	0.97
	AUCI	0.80	0.95	0.97
PZA	**Cmax**	1.00	0.95	0.93
	AUCT	0.93	0.94	0.96
	AUCI	0.93	0.93	0.96
ETB	**Cmax**	0.99	0.99	0.98
	AUCT	0.99	0.99	0.99
	AUCI	0.98	0.99	0.98

The analysis based on the Spearman correlation coefficient for the comparison of phase 1 and phase 2 shows that there is good agreement with each of the parameters of Cmax, AUCT and AUCI. This further confirms that the minimum time protocol proposed can be viewed as acceptable.

The results confirm that the comparison of AUCT (phase2) data to Cmax (phase1) yielded better results than that of AUCI (phase 2) vs Cmax (phase 1). The correlation values were 0.90, 0.88, 0.95, and 0.99 for the AUCT of INH, RIF, PZA and ETB respectively, and 0.86, 0.77, 0.93 and 0.98 respectively for AUCI. The use of the AUCT (phase 2) versus Cmax (phase 1) would allow for extrapolation using minimum times points to confirm Cmax estimation where regulatory approval has been achieved with extended 15 time point protocol. Comparison of Cmax (phase 2) to phase 1 AUCT and AUCI yields lower correlation coefficients for all drugs, the values being 0.87, 0.82, 0.93, and 0.99

for the AUCT of INH, RIF, PZA and ETB respectively, and 0.87, 0.80, 0.93 and 0.98 for AUCI of the 4 drugs, respectively. The results support the optimum minimum time points to be that of phase 2, namely 0, 0.25, 0.5, 1.0, 1.5, 2, 2.5, 3.0, 4.0, 6.0, 8.0.

5.2 Sub-Group analysis

Sub-group analysis was used to compare the pharmacokinetic parameters of individual drug formulations to fixed-dose combination drug formulations, healthy volunteers to patients and to compare the differences in pharmacokinetic parameters between the sexes. Phase 2 time protocol data was used in this part of investigation.

5.2.1 Comparison of individual to combined formulations

The comparison of the individual reference(R) data to that of the combined test (T) formulation for the 13 studies in the data set, aimed to reflect the overall comparative bioavailability of fixed-dose drug combination constituents, relative to the individual reference drug constituents.

Table 23 summarises this information using the pharmacokinetic parameters of Cmax, AUCT and AUCI. The table reflects the mean values and standard error of the mean (SEM) for the individual (reference) products and the combined products (test), respectively. The statistical tests used to determine significant differences were the paired t-test (Test 1) and the Wilcoxon matched pairs signed rank test (Test 2). Significance was declared when the p-values obtained were less than 0.05.

Table 23. Comparison of overall individual versus combined formulations

	Isoniazid (INH)					
	Cmax (mg/l)		AUCT (mg.h/l)		AUCI (mg.h/l)	
	R	T	R	T	R	T
Mean	6.06	5.61	21.93	21.70	27.14	27.24
SEM	0.21	0.19	0.79	0.75	1.06	1.06
Test 1	p=	<0.05	p=	0.59	P=	0.88
Test 2	P=	<0.05	P=	0.47	P=	0.73
	Rifampicin (RIF)					
	Cmax (mg/l)		AUCT (mg.h/l)		AUCI (mg.h/l)	
	R	T	R	T	R	T
Mean	13.03	11.98	51.60	49.33	67.96	67.17
SEM	0.31	0.29	1.29	1.17	1.92	1.99
Test 1	p=	<0.05	p=	<0.05	P=	0.59
Test 2	P=	<0.05	P=	0.05	P=	0.44
	Pyrazinamide (PZA)					
	Cmax (mg/l)		AUCT (mg.h/l)		AUCI (mg.h/l)	
	R	T	R	T	R	T
Mean	38.62	39.34	269.53	272.24	460.12	486.98
SEM	0.73	0.74	10.88	10.66	11.22	20.09
Test 1	p=	0.29	p=	0.43	P=	0.15
Test 2	P=	0.09	P=	0.35	P=	0.53
	Ethambutol (ETB)					
	Cmax (mg/l)		AUCT (mg.h/l)		AUCI (mg.h/l)	
	R	T	R	T	R	T
Mean	3.17	3.22	9.68	9.88	11.22	11.60
SEM	0.13	0.14	0.36	0.44	0.43	0.54
Test 1	p=	0.73	p=	0.64	p=	0.69
Test 2	P=	0.91	P=	0.94	P=	0.99

Table 23 concludes that Cmax values for INH and RIF were significantly higher for the reference product than the test. For INH these values were 6.06 mg/l and 5.61 mg/l for reference and test respectively. The AUCT values of INH 21.93 mg.h/l and 21.70 mg.h/l for the reference and test products respectively were not significantly different. The AUCI values, followed a similar trend. For RIF the values were 13.03 mg/l and 11.98 mg/l with significant difference for reference and test respectively. For RIF a similar trend to that of Cmax was observed for AUCT (51,60 mg.h/l and 49.33 mg.h/l) and AUCI (67.96 mg.h/l and 67.17 mg.h/l), but the differences were not significant. The Cmax , AUCT and AUCI values of PZA and ETB were similar for the reference and test values, with no significant differences between the pharmacokinetic values of the reference and test products.

The findings imply that although the formulations investigated were bioavailable isoniazid and rifampicin with concentrations generally within the recommended therapeutic range, there is a statistical significantly difference based on the overall data set investigated. The outcome shows that the fixed-dose drug combinations yield statistically significantly lower isoniazid and rifampicin levels than the individual (reference) formulations. This reduction might have serious implications for both the individual as well as for national tuberculosis control programmes due to potential for treatment failure and selection of drug-resistant mutants [109]. For pyrazinamide and ethambutol the results were comparable irrespective of whether the respective drugs were in individual (reference) or combined (test) formulations. This finding is reassuring for the prospect of fixed-dose drug formulations with respect to pyrazinamide and ethambutol.

5.2.2 Comparison of females versus males

The effect of sex on bioavailability was investigated. In the investigation healthy volunteers and patients were considered to be a common parameter. Table 24 summarises this information using the pharmacokinetic parameters of Cmax, AUCT and AUCI. The table reflects the mean values and standard error of the mean (SEM) for female and male results, respectively. The statistical tests used to determine significant differences were the paired t-test (Test 1) and the Wilcoxon matched pairs signed rank test (Test 2). Significance was declared where p-values obtained were less than 0.05.

Table 24. Comparative bioavailability in female and males overall data

	Isoniazid (INH)					
	Cmax (mg/l)		AUCT (mg.h/l)		AUCI (mg.h/l)	
	Female	Male	Female	Male	Female	Male
Mean	6.27	5.64	24.77	20.46	30.06	25.88
SEM	0.23	0.18	0.98	0.65	1.32	0.90
Test 1	p=	0.36	p=	0.05	p=	0.26
Test 2	P=	0.50	P=	0.11	P=	0.44
	Rifampicin (RIF)					
	Cmax (mg/l)		AUCT (mg.h/l)		AUCI (mg.h/l)	
	Female	Male	Female	Male	Female	Male
Mean	15.24	11.27	65.43	43.69	90.01	57.40
SEM	0.41	0.22	1.70	0.81	2.73	1.30
Test 1	p=	<0.05	p=	<0.05	p=	<0.05
Test 2	P=	<0.05	P=	<0.05	P=	<0.05
	Pyrazinamide (PZA)					
	Cmax (mg/l)		AUCT (mg.h/l)		AUCI (mg.h/l)	
	Female	Male	Female	Male	Female	Male
Mean	45.18	36.69	350.17	241.68	545.45	447.07
SEM	0.91	0.54	16.57	7.39	29.78	10.75
Test 1	p=	<0.05	p=	0.95	p=	<0.05
Test 2	P=	<0.05	P=	0.06	P=	<0.05
	Ethambutol (ETB)					
	Cmax (mg/l)		AUCT (mg.h/l)		AUCI (mg.h/l)	
	Female	Male	Female	Male	Female	Male
Mean	3.18	3.20	10.61	9.43	12.44	10.99
SEM	0.18	0.11	0.56	0.32	0.68	0.39
Test 1	p=	0.23	p=	0.95	p=	0.97
Test 2	P=	0.22	P=	0.99	P=	0.89

Table 24 concludes that females attained slightly higher Cmax values (6.27 mg/l) than males (5.64 mg/l) for INH. The differences were not significant. AUCT and AUCI values followed a similar trend in that female values were slightly higher but not significantly different.

RIF and PZA values were also marginally higher in females for all parameters (Cmax, AUCT and AUCI), with the exception of the AUCT for PZA, the differences were significant. The ETB values for Cmax were similar, for females (3.18 mg/l) and for males (3.20 mg/l) with no significant differences. The AUCT and AUCI values were also similar and not significantly different.

The findings show that females attain higher concentration of rifampicin and pyrazinamide that is statistically different to that of males. The observation becomes important in deciding on an effective dosing regimen to obtain a similar outcome in males and females. For isoniazid and ethambutol there was no statistical difference in the data and the values were comparable for males and females and within the recommended clinical range.

5.2.3 Comparison of healthy volunteers versus patients

The comparison investigated the effect of subjects being healthy volunteers (H) to that of being patients (P), regardless of sex status. The statistical tests applied to the bioavailability data were the unpaired t-test (Test 1) and the Mann Whitney non-parametric test (Test 2). The mean data of 471 healthy volunteers were compared to that of 126 patients. Table 25 summarises this information for the pharmacokinetic parameters of Cmax, AUCT and AUCI. The table reflects the mean values and standard error of the mean (SEM) for the results of healthy

volunteers and patients respectively. Significance was declared when the p-values obtained were less than 0.05.

The general trend in Table 25 shows that there is a significant difference when comparing the data. For INH and PZA healthy volunteers attained a lower Cmax than patients (Mean values for INH: 5.73 mg/l compared to 6.19 mg/l, respectively; For PZA: 38.62 mg/l compared to 39.85 mg/l respectively). This trend was consistent for AUCT and AUCI. The inverse was noted for RIF where patients mean values were lower; 8.61 mg/l compared to 13.45 mg/l.

This finding is almost certainly due to increased rifampicin metabolism caused by auto enzyme induction that would be experienced by patient subjects who would have received rifampicin prior to being on the clinical trial [42, 44, 51-53, 58, 92, 100]. All these results were statistically significant. The values were within clinical range.

No studies were done, where ETB was administered to patients hence the comparison to healthy volunteers could not be determined within the realm of the studies investigated.

The results confirm the two distinct groups of subjects, that is healthy volunteers and patients. It further endorses the fact that the results of clinical investigations of antituberculosis drug bioavailability, done in healthy volunteers will differ from those if similar studies are done in patients. This has important relevance in particular when outcomes are to be extrapolated or interpolated from healthy volunteers to patients and vice versa.

Table 25. Comparison of overall healthy volunteers(H) to patients (P) overall data

	Isoniazid (INH)					
	Cmax (mg/l)		AUCT (mg.h/l)		AUCI (mg.h/l)	
	H	P	H	P	H	P
Mean	5.73	6.19	21.77	21.96	27.27	26.93
SEM	0.16	0.32	0.59	1.31	0.81	1.80
Test 1	p=	<0.05	p=	<0.05	p=	<0.05
Test 2	P=	<0.05	P=	<0.05	P=	0.10
	Rifampicin (RIF)					
	Cmax (mg/l)		AUCT (mg.h/l)		AUCI (mg.h/l)	
	H	P	H	P	H	P
Mean	13.45	8.61	55.43	30.01	75.78	33.69
SEM	0.23	0.32	0.92	1.03	1.46	1.19
Test 1	p=	<0.05	p=	<0.05	p=	<0.05
Test 2	P=	<0.05	P=	<0.05	P=	<0.05
	Pyrazinamide (PZA)					
	Cmax (mg/l)		AUCT (mg.h/l)		AUCI (mg.h/l)	
	H	P	H	P	H	P
Mean	38.62	39.85	293.70	215.65	494.50	422.65
SEM	0.65	0.83	10.16	3.91	14.17	18.20
Test 1	p=	<0.05	p=	0.08	p=	<0.05
Test 2	P=	<0.05	P=	<0.05	P=	<0.05

5.3 Residual maximum likelihood analysis

In support of the findings of the correlation and sub-group analysis as reflected in Table 21-25, residual maximum likelihood analysis (REML) was used to fit linear mixed models to the logarithm differences of the pharmacokinetic parameter AUCT of the 4 drugs investigated [36-38]. Based on the pharmacokinetic parameters Cmax, AUCT and AUCI that were investigated the parameter AUCT was found to be better than AUCI with respect to correlation analysis. The basis for choosing AUCT for the (REML) analysis thus takes into consideration absorption and elimination aspects of each of the drugs investigated. The logarithm of the differences would therefore produce the necessary sensitivity to detect any comparative differences using all data as a collective data set. The REML model that was established thus consisted of two components. The fixed model took into account a constant parameter, sex status, whether the subjects were healthy volunteers or patients, and whether the formulation used was reference individual drug(s) or test fixed-dose drug combination. The second component was the random model, that investigated the various studies undertaken and subjects within the studies [176].

Table 26. REML variance analysis components

Drug	No of Units	Stratum Variances			Walden test for Fixed effect (p-values)		
		Study	Study Subjects	Units	Male/ Female	Healthy/ Patient	Reference/ Test
INH	496	10	235	247	0.580	0.545	0.472
RIF	578	12	276	286	<0.001	<0.001	0.338
PZA	212	3	100	105	0.014	<0.001	0.188
ETB	140	-	68	69	0.144	-	0.265

REML analysis concluded that with respect to isoniazid there was no effect due to sex. Similarly no effect was found whether the subject was a healthy volunteer or a patient; and no effect was confirmed by the formulation composition. For rifampicin REML confirmed that there was no effect due to formulation, but the sex of the subject and whether the subject was a healthy volunteers or patient had effect. Pyrazinamide showed no effect due to formulation, but being male or female, and whether the subject was a healthy volunteer or patient subject, contributed an effect. For ethambutol only the parameters of sex and formulation were investigated as no studies, that were investigated were done in patients. No effect was registered for the parameters of sex and formulation.

5.4 Subject Reduction analysis

Subject reduction was investigated to test for the minimum number of subjects required for bioavailability and bioequivalence testing. The optimum minimum time protocol as determined in Chapter 4 was employed, using Study 13 as an example, as it showed good bioavailability and bioequivalence results across all minimum time protocols investigated.

A random process of deselecting was used to eliminate subjects from the original randomisation sequence to ensure that similar numbers remained in each sequence of reference/test (RT) and test/reference (TR) arms of the study respectively. The effect of subject numbers of 24, 18 and 12 subjects is presented in Table 27.

Table 27. Randomised deselecting of subjects

Subjects	24	18	12
R/T	1 2 7 8 9 11 12 14 18 21 23 24	1 2 7 8 12 14 21 23 24	2 7 8 21 23 24
T/R	3 4 5 6 10 13 15 16 17 19 20 22	4 5 10 15 16 17 19 20 22	4 10 17 19 20 22

Tables 28, 29 and 30 represent the bioavailability data for 24, 18, and 12 subjects. The tables present the pharmacokinetic parameters Tmax, Cmax, Half-life, AUCT and AUCI for reference and test formulations. Mean and standard error of the mean (SEM) were used. The units of measurement for each of the respective parameters are indicated in brackets; Tmax (h), Cmax (mg/l), Half–life (h), AUC and AUCI (mg.h/l). The parametric Students–t-Test (Test 1) and non-parametric Wilcoxon Signed Rank test (Test 2) were used to determine statistical significance between the reference and test formulations. Significance was declared where the p – values obtained were less than 0.05 when comparing the reference to the test.

Tables 31, 32 and 33 represent the bioequivalence data for 24, 18 and 12 subjects respectively. For bioequivalence testing the parameters of importance, i.e. Cmax, AUC and AUCI were summarised for each drug with respect to the point estimate, test/ reference ratio, the percentage span, (the difference between the upper and lower confidence interval), and the most important parameter in the

assessment, the 90% confidence interval. Where the required data falls within the confidence interval (80-125%) the data is presented in bold and when not within the required parameters it is presented as ordinary text.

Table 28. Bioavailability results of optimum protocol for 24 subjects

Study 13	Isoniazid (INH)									
N=24	Tmax (h)		Cmax (mg/l)		T1/2 (h)		AUCT (mg.h/l)		AUCI (mg.h/l)	
HV	R	T	R	T	R	T	R	T	R	T
Mean	1.19	1.30	4.66	4.18	3.54	3.14	17.12	17.01	22.88	21.33
SEM	0.16	0.14	0.38	0.29	0.31	0.13	1.35	1.00	2.10	1.30
Test 1	P=	0.50	P=	0.12	P=	0.17	P=	0.92	P=	0.30
Test 2	P=	0.52	P=	0.17	P=	0.67	P=	0.98	P=	0.38
	Rifampicin (RIF)									
	Tmax (h)		Cmax (mg/l)		T1/2 (h)		AUCT (mg.h/l)		AUCI (mg.h/l)	
	R	T	R	T	R	T	R	T	R	T
Mean	2.08	2.25	11.74	10.75	3.69	4.00	50.58	46.07	70.08	68.17
SEM	0.12	0.15	0.68	0.53	0.19	0.66	2.96	2.29	4.51	6.30
Test 1	P=	0.19	P=	0.10	P=	0.60	P=	0.06	P=	0.71
Test 2	P=	0.24	P=	0.09	P=	0.22	P=	0.07	P=	0.51
	Pyrazinamide (PZA)									
	Tmax (h)		Cmax (mg/l)		T1/2 (h)		AUCT (mg.h/l)		AUCI (mg.h/l)	
	R	T	R	T	R	T	R	T	R	T
Mean	2.46	1.90	37.59	37.89	9.73	8.59	209.1	219.0	545.1	497.8
SEM	0.17	0.21	1.47	1.42	0.65	0.55	8.63	7.69	36.67	30.00
Test 1	P=	<.05	P=	0.77	P=	0.09	P=	0.07	P=	0.16
Test 2	P=	<.05	P=	0.86	P=	0.34	P=	<.05	P=	0.65
	Ethambutol (ETB)									
	Tmax (h)		Cmax (mg/l)		T1/2 (h)		AUCT (mg.h/l)		AUCI (mg.h/l)	
	R	T	R	T	R	T	R	T	R	T
Mean	2.96	2.83	2.60	2.63	2.23	2.38	9.23	9.15	10.72	10.86
SEM	0.20	0.16	0.22	0.21	0.12	0.12	0.79	0.80	0.95	0.95
Test 1	p=	0.64	P=	0.88	P=	0.37	P=	0.92	P=	0.90
Test 2	P=	0.81	P=	0.93	P=	0.27	P=	0.74	P=	0.80

Table 29. Bioavailability results of optimum protocol for 18 subjects

Study 13	Isoniazid (INH)									
N=18	Tmax (h)		Cmax (mg/l)		T1/2 (h)		AUCT (mg.h/l)		AUCI (mg.h/l)	
HV	R	T	R	T	R	T	R	T	R	T
Mean	1.08	1.38	4.86	4.27	3.46	3.06	16.90	17.02	22.28	21.18
SEM	0.17	0.16	0.48	0.36	0.38	0.15	1.65	1.25	2.51	1.65
Test 1	P=	0.12	P=	0.13	P=	0.24	P=	0.93	P=	0.54
Test 2	P=	0.10	P=	0.19	P=	0.72	P=	0.89	P=	0.69
	Rifampicin (RIF)									
	Tmax (h)		Cmax (mg/l)		T1/2 (h)		AUCT (mg.h/l)		AUCI (mg.h/l)	
	R	T	R	T	R	T	R	T	R	T
Mean	2.11	2.31	12.05	11.11	3.56	3.36	52.15	46.15	70.71	63.77
SEM	0.14	0.18	0.77	0.62	0.22	0.29	3.60	2.78	5.58	5.48
Test 1	P=	0.22	P=	0.12	P=	0.25	P=	0.03	P=	0.13
Test 2	P=	0.25	P=	0.13	P=	0.24	P=	0.05	P=	0.29
	Pyrazinamide (PZA)									
	Tmax (h)		Cmax (mg/l)		T1/2 (h)		AUCT (mg.h/l)		AUCI (mg.h/l)	
	R	T	R	T	R	T	R	T	R	T
Mean	2.53	1.89	38.19	38.90	10.04	8.59	214.7	223.4	565	511.0
SEM	0.18	0.22	1.79	1.63	0.80	0.71	10.63	9.17	40.44	38.25
Test 1	P=	<.05	P=	0.58	P=	0.08	P=	0.22	P=	0.19
Test 2	P=	<05	P=	0.62	P=	0.16	P=	0.11	P=	0.45
	Ethambutol (ETB)									
	Tmax (h)		Cmax (mg/l)		T1/2 (h)		AUCT (mg.h/l)		AUCI (mg.h/l)	
	R	T	R	T	R	T	R	T	R	T
Mean	2.94	2.81	2.50	2.79	2.19	2.31	9.23	9.85	10.50	11.64
SEM	0.26	0.13	0.27	0.27	0.15	0.12	0.94	0.97	1.08	1.16
Test 1	p=	0.67	P=	0.33	P=	0.51	P=	0.58	P=	0.40
Test 2	P=	0.91	P=	0.34	P=	0.36	P=	0.62	P=	0.54

Table 30. Bioavailability results of optimum protocol for 12 subjects

Study 13	Isoniazid (INH)									
N=12	Tmax (h)		Cmax (mg/l)		T1/2 (h)		AUCT (mg.h/l)		AUCI (mg.h/l)	
HV	R	T	R	T	R	T	R	T	R	T
Mean	1.10	1.50	4.62	4.00	3.09	2.91	15.78	16.65	20.23	20.39
SEM	0.21	0.20	0.45	0.36	0.36	0.18	1.22	1.58	2.03	2.03
Test 1	P=	0.12	P=	0.25	P=	0.57	P=	0.54	P=	0.93
Test 2	P=	0.10	P=	0.38	P=	0.97	P=	0.62	P=	0.97
	Rifampicin (RIF)									
	Tmax (h)		Cmax (mg/l)		T1/2 (h)		AUCT (mg.h/l)		AUCI (mg.h/l)	
	R	T	R	T	R	T	R	T	R	T
Mean	2.21	2.42	12.51	10.63	3.35	2.96	52.89	44.07	70.26	57.71
SEM	0.16	0.23	0.96	0.66	0.14	0.22	4.80	3.42	7.13	5.76
Test 1	P=	0.34	P=	0.01	P=	0.08	P=	0.01	P=	0.02
Test 2	P=	0.44	P=	0.01	P=	0.09	P=	0.02	P=	0.03
	Pyrazinamide (PZA)									
	Tmax (h)		Cmax (mg/l)		T1/2 (h)		AUCT (mg.h/l)		AUCI (mg.h/l)	
	R	T	R	T	R	T	R	T	R	T
Mean	2.50	2.04	39.29	38.98	9.16	7.14	215.8	223.0	529.8	447.9
SEM	0.20	0.29	2.31	2.30	0.86	0.34	14.58	11.71	46.51	26.16
Test 1	P=	0.09	P=	0.85	P=	<.05	P=	0.44	P=	0.05
Test 2	P=	0.15	P=	0.91	P=	<.05	P=	0.23	P=	0.11
	Ethambutol (ETB)									
	Tmax (h)		Cmax (mg/l)		T1/2 (h)		AUCT (mg.h/l)		AUCI (mg.h/l)	
	R	T	R	T	R	T	R	T	R	T
Mean	3.04	2.75	2.42	2.74	2.22	2.41	8.78	9.58	9.92	11.41
SEM	0.38	0.20	0.34	0.33	0.21	0.15	1.06	1.14	1.12	1.33
Test 1	p=	0.56	P=	0.44	P=	0.50	P=	0.56	P=	0.37
Test 2	P=	0.81	P=	0.52	P=	0.42	P=	0.57	P=	0.47

Table 31. Bioequivalence results of optimum protocol for 24 subjects

Study 13 N=24 HV	Median/ Point Estimate	Test/ Reference Ratio	90% Confidence Interval	% Span
Isoniazid (INH)				
Cmax	92.81	0.97	**80.29-105.05**	22.76
AUC	102.57	1.08	**92.33-113.82**	21.49
AUCI	97.84	1.04	**88.06-111.99**	23.93
Rifampicin (RIF)				
Cmax	91.52	0.95	**85.51-100.82**	15.30
AUC	91.86	0.94	**86.29-98.43**	12.13
AUCI	91.48	0.98	**85.74-100.39**	14.65
Pyrazinamide (PZA)				
Cmax	100.21	1.02	**95.93-106.00**	10.07
AUC	106.33	1.06	**102.72-109.17**	6.45
AUCI	94.81	0.96	**82.68-105.83**	23.14
Ethambutol (ETB)				
Cmax	100.39	1.15	**84.1-118.53**	34.40
AUC	91.79	1.11	**82.83-111.66**	28.83
AUCI	94.77	1.16	**82.16-117.13**	34.97

Table 32. Bioequivalence results of optimum protocol for 18 subjects

Study 13 N=18 HV	Median/ Point Estimate	Test/ Reference Ratio	90% Confidence Interval	% Span
Isoniazid (INH)				
Cmax	91.58	0.96	**80.67-104.70**	24.04
AUC	105.08	1.11	**89.31-118.20**	28.90
AUCI	99.33	1.07	**87.21-114.11**	26.90
Rifampicin (RIF)				
Cmax	91.52	0.95	**85.75-100.43**	14.69
AUC	89.52	0.91	**83.25-95.55**	12.30
AUCI	88.53	0.92	**83.26-95.19**	11.94
Pyrazinamide (PZA)				
Cmax	102.13	1.03	**96.60-108.79**	12.19
AUC	106.33	1.06	**100.94-110.13**	9.19
AUCI	91.50	0.94	**80.01-105.76**	25.75
Ethambutol (ETB)				
Cmax	110.53	1.27	89.72-138.34	48.61
AUC	104.68	1.21	84.59-127.62	43.03
AUCI	103.58	1.27	87.75-133.97	46.22

Table 33. Bioequivalence results of optimum protocol for 12 subjects

Study 13 N=12 HV	Median/ Point Estimate	Test/ Reference Ratio	90% Confidence Interval	% Span
Isoniazid (INH)				
Cmax	89.90	0.94	71.47-116.38	44.91
AUC	105.49	1.10	90.18-126.13	35.94
AUCI	101.07	1.09	82.40-126.85	44.45
Rifampicin (RIF)				
Cmax	85.76	0.87	**80.21-95.11**	14.90
AUC	85.67	0.86	77.88-93.32	15.45
AUCI	82.99	0.85	76.85-88.61	11.75
Pyrazinamide (PZA)				
Cmax	99.94	1.00	**90.72-108.12**	17.39
AUC	106.63	1.06	**98.96-111.05**	12.08
AUCI	86.39	0.89	74.52-101.87	27.35
Ethambutol (ETB)				
Cmax	113.47	1.33	84.52-156.22	71.70
AUC	105.00	1.20	80.70-135.15	54.45
AUCI	103.58	1.27	81.53-148.63	67.10

For all drugs, Cmax values fell within the therapeutic range whether 24, 18 and 12 were included in the analysis. The results were not significantly different when a reduced sample size was used with the exception of RIF. When the sample size was reduced to 12 subjects, the Cmax values of rifampcin were significantly different to those when 24 subjects were included in the analysis.

The AUCT and AUCI parameters of rifampicin were also significantly different when 12 subjects were used in the investigation. This shows deviation from the overall template that is fairly consistent for 24 and 18 subjects, respectively.

The results indicate that one could potentially utilise 12 subjects, to investigate the bioavailability, although deviation could be anticipated relative to the current 24 subjects testing protocol, for RIF in particular. On the side of caution in view of the current investigation it is evident that 18 subjects would be acceptable.

For bioequivalence all the values for the pharmacokinetic parameters Cmax, AUCT and AUCI fell within the 90% confidence interval when 24 subjects were included. Reducing the subject number to 18 as described, the results compared favourably and were within the confidence interval for INH, RIF and PZA for parameters Cmax, AUCT and AUCI, respectively. This was not the case for ethambutol whose confidence interval fell outside the range for all parameters.

Reducing the subject number to 12 as described, the following parameters complied with the necessary confidence interval: for rifampicin, only Cmax; for pyrazinamide, Cmax and AUCT. The interval for isoniazid fell marginally outside of the upper end of the required range for AUCT and AUCI. The other pharmacokinetic parameters were all outside the confidence interval, and all parameters fell outside of the confidence interval for ethambutol.

In conclusion the investigation into reduction of subject number in the optimum minimum time points protocol has revealed that within the context of the selected four drug fixed-dose combination study indicated, that the total number of subjects in a study could potentially be reduced from the initial 24 subjects to 12 subjects. The values attained would still be within the stipulated clinical range when comparing the pharmacokinetic parameters of importance Cmax, AUCT and AUCI. These pharmacokinetic parameters were also not significantly different. The exception was RIF that was within clinical range, but the comparison showed the results to be significantly different for the pharmacokinetic parameters Cmax, AUCT and AUCI.

With respect to bioequivalence whilst utilising 24 subjects and the optimum minimum time points protocol, all drugs and all pharmacokinetic parameters were within the confidence interval. For 18 subjects only ethambutol was not within the confidence interval but all the other drugs INH, RIF and PZA were within the confidence interval. This indicates, that potentially FDC's with the latter combination could be investigated with 18 subjects and the optimum minimum time protocol of phase 2 (with sampling times at 0, 0.25, 0.5, 1.0, 1.5, 2, 2.5, 3.0, 4.0, 6.0 and 8.0 hours) would be adequate. The exception would be ETB, that would be marginally out with respect to AUC. For 12 subjects some of the parameters complied with the confidence interval while others were marginally on the outside. The confidence interval values for Cmax, AUCT and AUCI of ethambutol were not within the necessary limits. The overall picture does not yield the desired confidence interval in comparison to that of 24 and 18 subjects investigated.

The limiting analysis with respect to bioavailability and bioequivalence testing to determine the reduced subject number that would be appropriate for fixed-dose drug combinations, is in fact the bioequivalence analysis. The bioequivalence analysis is of most relevance in evaluating the suitability of new formulations (example FDCs) to replace those in established use (represented by the reference product). Therefore for four drug combinations it would be apparent that 24 subjects would be the minimum, if given the overview as sketched and the results obtained. For three and two drug FDCs of INH, RIF, and PZA (excluding ETB), 18 subjects could be appropriate.

Chapter 6
Conclusion

The objective of the study was to investigate a number of modified screening-time protocols used for bioavailability and bioequivalence studies of anti-tuberculosis fixed-dose drug combination formulations to determine the minimum time points that are required to confirm bioavailability and bioequivalence relative to an existing 15 time point protocol (0, 0.25, 0.5, 1.0, 1.5, 2, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0, 36.0, 48.0 hours).

The design of all the studies considered in this thesis complied with South African national drug regulatory authority requirements for the determination of comparative bioavailability and bioequivalence testing of fixed-dose drug formulations. The following parameters for bioavailability were compared using concentration-time curves: Tmax, Cmax, Half-life, AUCT and AUCI. For bioequivalence, the parameters Cmax, AUCT and AUCI were used. This data is presented in Chapter 3 as the extended time protocol results and was used as the basis for the comparative to determine the optimum minimum time schedule. With the exception of study 9 the outcome of the 13 studies investigated, indicates that all the studies provide sufficient evidence of comparable bioavailability and bioequivalence using the pharmacokinetic parameters and the relevant criteria as set out in Chapter 2. The results and conclusions from all the studies formed the basis for the conclusions that follow.

In Chapter 4, the results obtained in Chapter 3 using the extended 15-time point protocol, were compared to a modified minimum time points protocol containing fewer time points with respect to bioavailability and bioequivalence testing. The comparative correlation analysis on the pharmacokinetic parameters of Cmax, AUCT and AUCI investigated were also used to confirm the optimum minimum time points. Eleven time points, namely 0, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, and 8

hours, were sufficient for demonstration of comparative bioavailability and bioequivalence of INH, RIF, PZA and ETB. A schedule of 6 time points, namely at 0, 1, 2, 4, 6 and 8 hours, is not sufficiently reliable for determining the bioavailability and bioequivalence of anti-tuberculosis fixed-dose drug formulations. This is particularly relevant when Cmax and AUCI parameters were compared in the reference and test formulations.

In Chapter 5 the objective was to process and investigate the number of fixed-dose drug formulations as an inclusive data set, for comparison of individual drugs to combined formulations, sex, and disease status.

When the data for all the studies was pooled, the following was observed: for healthy volunteers and patients, for sub-group analysis, compared individual drugs to combined formulations, showed that Cmax had significantly higher values for the reference individual products than for the combined test products, in respect of INH and RIF, but not for PZA and ETB. There were no significant differences in either AUCT or AUCI.

Cmax, AUCT and AUCI for INH, RIF and PZA, respectively, showed significant differences between healthy volunteers and patients. Healthy volunteers attained higher Cmax values for RIF than patients. Patients attained higher Cmax values for INH and PZA than healthy volunteers.

Bioavailability of the tested anti-tuberculosis agents differed according to the study subjects being investigated, and clear differences were found between healthy volunteers and patients. It is thus important not to assume that similar outcomes will be achieved when doing studies in either patients or healthy volunteers.

Females attained significantly higher Cmax and AUCI values for RIF and PZA respectively, when compared with males. It might therefore be important to formulate a specific fixed-dose drug treatment regimen for males and females respectively, with particular reference to RIF and PZA. There were no differences between females and males with regard to the pharmacokinetic parameters INH and ETB, respectively. Residual maximum likelihood analysis using linear mixed models, fitted for the differences between AUCs, provides supporting evidence for these conclusions.

Subject reduction analysis investigated the optimum number of subjects required for bioavailability and bioequivalence assessment of a fixed-dose drug formulation. In testing for bioequivalence of four-drug fixed-dose anti-tuberculosis drug combinations, more than 18 subjects are required. The current practice of striving to enrol 24 subjects in a clinical trial to determine the comparative quality of a fixed-dose drug combination would therefore need to be retained.

A validated bioavailability and bioequivalence protocol which uses optimum minimum time points has been achieved. This reduces development time and cost, and simplifies regulatory requirements for INH, RIF, PZA and ETB of the fixed-dose drug combinations investigated. The modified protocol will not compromise existing standards and policy.

The minimum time points schedule also implies that less specimens are needed for pharmacokinetic trial(s) time-concentration profiles for the investigation of FDCs. This will bring about a potential saving of time to determine the outcome or continued measure of the quality of an existing FDC in the market with respect to post- marketing surveillance.

Whilst the benefit should be apparent for the larger pharmaceutical companies who would be the predominant role players, by already having FDCs in the

market place, smaller pharmaceutical companies might find the modified protocol attractive enough to engage the process of producing FDCs with the rigors of regulatory authority requirements, thereby contributing to the need for the manufacture of fixed-dose drug formulations.

These findings can further contribute to the WHO and partners, relevant information to support the four-drug FDC tablet in the WHO Model List of Essential Drugs. Thus the modified and simplified minimum time points schedule protocol as proposed, could become the policy of choice.

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Appendices

Appendix 1

Information and Consent form example

SUBJECT INFORMATION AND CONSENT FORM

The bioavailability and bioequivalence of rifampicin, ethambutol, isoniazid and pyrazinamide as a single dose in a fixed dose combination formulation, compared to equivalent doses of single drug reference preparations of the four drugs.

Background and Study objectives. The fixed dose combination (FDC) tablet containing rifampicin 150 mg, ethambutol 275 mg, isoniazid 75 mg and pyrazinamide 400 mg has been developed recently by Novartis for the treatment of patients with tuberculosis. The administration of drugs in FDCs for tuberculosis has certain advantages for drug distribution and administration. However, it is necessary to prove that the drugs reach blood concentrations in humans equivalent to those achieved by drugs known to be of good efficacy, safety and quality.

The aim of this study is to evaluate the bioavailability of the new FDC compared to that of equivalent doses of single drug reference products, and to determine whether the new product is bioequivalent.

Study design and performance. This study will consist of 2 treatment periods of 48 hours each. At each treatment period you will take either the FDC or the reference tablets. On each occasion you will take a total dose of 600 mg of rifampicin, 1100 mg of ethambutol, 300 mg of isoniazid and 1500 mg or 1600 mg of pyrazinamide. The drug administrations will be separated by 1 week.

Description of screening procedures (within 4 weeks before the study). Screening will take place in the Department of Pharmacology (K Floor, Old Main Building, Groote Schuur Hospital) by prior arrangement with Irene Bloch.

Screening will include a physical examination, blood pressure and heart rate measurement, as well as routine blood and urine tests, screening for recreational drugs, tests for HIV and hepatitis B. Pre-test counselling which includes information regarding the HIV virus /AIDS, way of spreading, risk factors and consequences will be provided verbally by the screening doctor prior to blood test. For the individuals testing positive, follow-up will be made available at Groote Schuur Hospital HIV clinic which includes access to both doctors and social workers.

Approximately 25ml of blood will be collected and a small urine sample.

You <u>will be</u> included in the study if:

- You are a healthy volunteer, aged between 18 and 55 years.
- Your weight is within the range dictated by body mass indices.
- Your clinical history, examination and laboratory values are normal, as assessed by the clinical investigator.
- Your blood pressure and heart rate are normal.
- You signed informed consent to participate in the study.

You <u>will not</u> be included in the study if the following is recorded:

Any illness or conditions considered by the medical doctor and/or described in the study protocol as a reason for excluding you from the study (examples: high blood pressure, cancer, etc.).

- History of hypersensitivity to a drug(s).
- Active allergic disease or a history of significant allergic disease.
- History of drug or alcohol addiction.
- Consumption of large quantities of coffee, tea, alcohol and wine.
- Blood donation of more than 500ml during the previous 6 months.
- Positive test for HIV and hepatitis B.
- Are a smoker or have smoked at all in the 3 months prior to the study.
- Use of any drugs or medication (including the oral contraceptive) within 2 weeks of the study, or within a month of the study in the case of certain medications.
- Are pregnant or at risk of becoming pregnant.
  Description of each treatment period. You will be required to report to the Pharmacology Department on each morning before the drug dosing days at 08h00.

You are required to meet at "admissions" (on E Floor at the main entrance) of Groote Schuur Hospital at 20h00 on the evening preceding each dosing day. You will be admitted to ***ward G13*** before receiving a light supper. You will fast overnight from 22h00 and you will continue to fast until 10h00 the following day when a light standardised breakfast will be served. A light standardised lunch will be served at 13h00 and a standardised supper will be served at 18h00. Further refreshments will also be available after lunch.
On the morning of the dosing day a venous cannula will be inserted into a suitable arm vein. Through this cannula blood samples will be collected for the first 12 hours after medication.

Before receiving the first dose, you will have the following procedures:

- Measurement of blood pressure, heart rate and temperature.

You will receive treatments with water sitting in an upright position. ***You will remain in the sitting position for another 30 minutes and thereafter semi-recumbent until 2 hours after each treatment.***
Blood samples will be collected for drug assays at the following times, before medication, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 36and 48 hours after drug administration.
You will leave the unit after the 12 hour blood sample, but will be required to report to the Department of Pharmacology at 07h55 the following day for the 24hr blood sample, again at 19h55 for the 36 hour sample and at 07h55 on the second morning after drug administration for the 48 hour sample.

During the second treatment period, the above mentioned procedures will be repeated.
You will be required to report to the Pharmacology Department at 08h00 for further blood tests, 6 days after the last drug administration. These are "safety tests" to detect the unlikely event of damage to your liver, kidneys or blood after exposure to the study drugs. Not more than 340ml of blood will be withdrawn during the study for the drug assays and laboratory tests.

Subject's obligation. Please follow the requirements listed below:

- ***YOU ARE OBLIGED TO CONSUME COMPLETELY THE MEALS PROVIDED.*** You are only allowed to eat the standardised meals and refreshments provided, this is to limit individual variation in the absorption and metabolism of the study drugs.

- You are ***not allowed to take any non trial medication***, including over-the-counter remedies throughout the study and in the week before the study without consulting the investigator in advance.
- ***No strenuous physical activity*** may be performed from 48 hours before and until 48 hours after each administration.
- ***No alcohol*** or ***caffeine*** may be consumed from 48 hours before and until 48 hours after each administration.

Withdrawal from the study. It is the privilege of any subject to withdraw from the study. Subjects must agree to obey the instructions of the investigator concerning matters pertaining to the health of the individual after drug administration. The clinical investigator may withdraw subjects from the study for clinical reasons or protocol violations by the subject. Protocol violation is defined as wilful disobedience of instructions communicated verbally or in writing.

Subject remuneration. Compensation for time-loss and inconvenience suffered as a result of participation in the study will be made on completion of the study. Subjects who do not complete the study for *bona fide* reasons or other clinical considerations will be compensated according to the amount of time lost and inconvenience suffered i.e. on a_pro rata basis. Protocol violation may result in forfeiture of remuneration.

Information on adverse events. Please report any unusual symptoms or deterioration in your health to the investigators immediately. The TB treatment which you will be taking has been associated with the following adverse events: adverse events: loss of appetite, nausea, vomiting, abdominal discomfort, rashes, joint pains and headache are relatively common (1/10 to 1/100). Uncommon effects include hepatitis (liver damage), peripheral neuritis, "flu-like syndrome", hypersensitivity, blood dyscrasias (damage to the blood cells), nephrotoxicity (kidney damage), neurotoxicity and optic neuritis. Rifampicin may produce an orange-red discolouration of body fluids; *contact lenses should not be worn until 24 hours after each drug administration.*

Subject's information. Participation in this study is entirely voluntary, you are not obliged to take part. If you decide to take part you will need to sign to say that you have given your consent to participate. If you agree to participate, you may withdraw from the study at any time, without prejudice to you for doing so. The personal information obtained about you during the course of this study will remain confidential and in reporting the results of the study, you will be referred to only by a code number.

In accordance with legal requirement, during the course of this clinical trial, you will be covered by an insurance policy.

You will be given a copy of this information sheet and consent form, and may ask the following contact persons for additional information, at any time during the study:

Name	Telephone (Home)	Telephone (Work)
Irene Bloch	021-551 1039	021-406 6148/ 406 6292
Ms Alicia Evans	021-448 3287	021-406 6295

VOLUNTEER CONSENT FORM

1. I agree to take part in the trial of the bioavailability of a fixed dose combination tablet containing rifampicin, ethambutol, isoniazid and pyrazinamide.
2. I confirm that the Supervising Doctor, _____ has given me a full explanation of the nature, purpose and duration of the study and of what I shall be expected to do and has advised me about the ill-effects, including discomfort or inconvenience, to my health or well-being that he believes might result from participation. I have also read an information document relating to the study.
3. I have been informed that an insurance policy has been taken out for this study.
4. I have been given the opportunity to ask any questions concerning the study and I have to my satisfaction understood the supervising doctor's explanation and advice.
5. I have informed the supervising doctor of all my previous or present illnesses, together with any medication/drugs, of whatever nature, I have taken in the last month, or am taking, or am planning to take, whether prescribed or not, and of any consultation that I have had with my doctor in the last months, whether or not it resulted in medication or drug therapy.
6. I have further informed the supervising doctor of any participation by me in other volunteer studies in the past year.
7. I confirm that I do not fulfil any of the exclusion criteria for this study as described in the volunteer information document.
8. I agree to comply in good faith with all instructions given to me by the supervising doctor and undertake to notify him at once if I suffer any unexpected and unusual symptoms or any deterioration whatsoever in my health or well-being however caused.
9. I am aware that this study has been subjected to ethics review by an Ethics Committee.
10. I agree to undergo blood tests as described in the subject information document, including a test for the AIDS virus.
    I understand that the following procedures will be carried out as part of the study:
    a) Administration of the study drugs on 2 occasions.
    b) The withdrawal of blood specimens during the study periods as described in the subject information document.
11. I understand that I am free to withdraw my consent to participate in the study at any time without the need to justify my decision, but I confirm that while participating in the study I will not knowingly do anything that I might reasonably assume could affect the results of it. I further understand that any information that becomes available during the course of the study that may affect my willingness to take part will be disclosed to me as soon as practicable.
12. On the basis that my name in connection with this study will not be disclosed to any person other than the Sponsor or Regulatory Authority, I do not claim to be entitled to restrict in any way the use to which the results of the study may

be put. In particular, I agree to disclosure of any report of those results to Regulatory Authorities for medicines.

13. I understand that representatives of the Company or Regulatory Authorities may wish to inspect my medical records to verify the information collected. By signing this document I give permission for this review of my records. I am aware that, although this study may help the advance of medical knowledge, I will not directly receive health benefits from it, and that if I have not been truthful in any of the information I have supplied, or if I do not fully comply with the directions given to me, I may harm myself by participating in the study.

I confirm that I have received a copy of this Consent Form and of the Volunteer Information sheet relating to the study.

Last Name First Name

....

Date...../...../.....

Signature of volunteer

.....

Place/Date ..../...../.....

I confirm that I have explained the nature, purpose and foreseeable effects of the trial to the volunteer whose name is printed above, and that he has consented to participate.

The volunteer confirmed consent by signing above.

Name of supervising doctor

.....

Date ...../...../.....

(please print)

Signature of supervising doctor

.....

Appendix 2

Data files

Attached are data files of the 13 respective studies that can be opened in WinNonlin Standard Edition Version 1.5 (WinNonlin © 1984-1997, Scientific Consulting, Inc) or Microsoft ® Excel 97 SR-2 (Excel © 1985-1997 Microsoft Corporation) using the comma delimiter function to get access to the data.

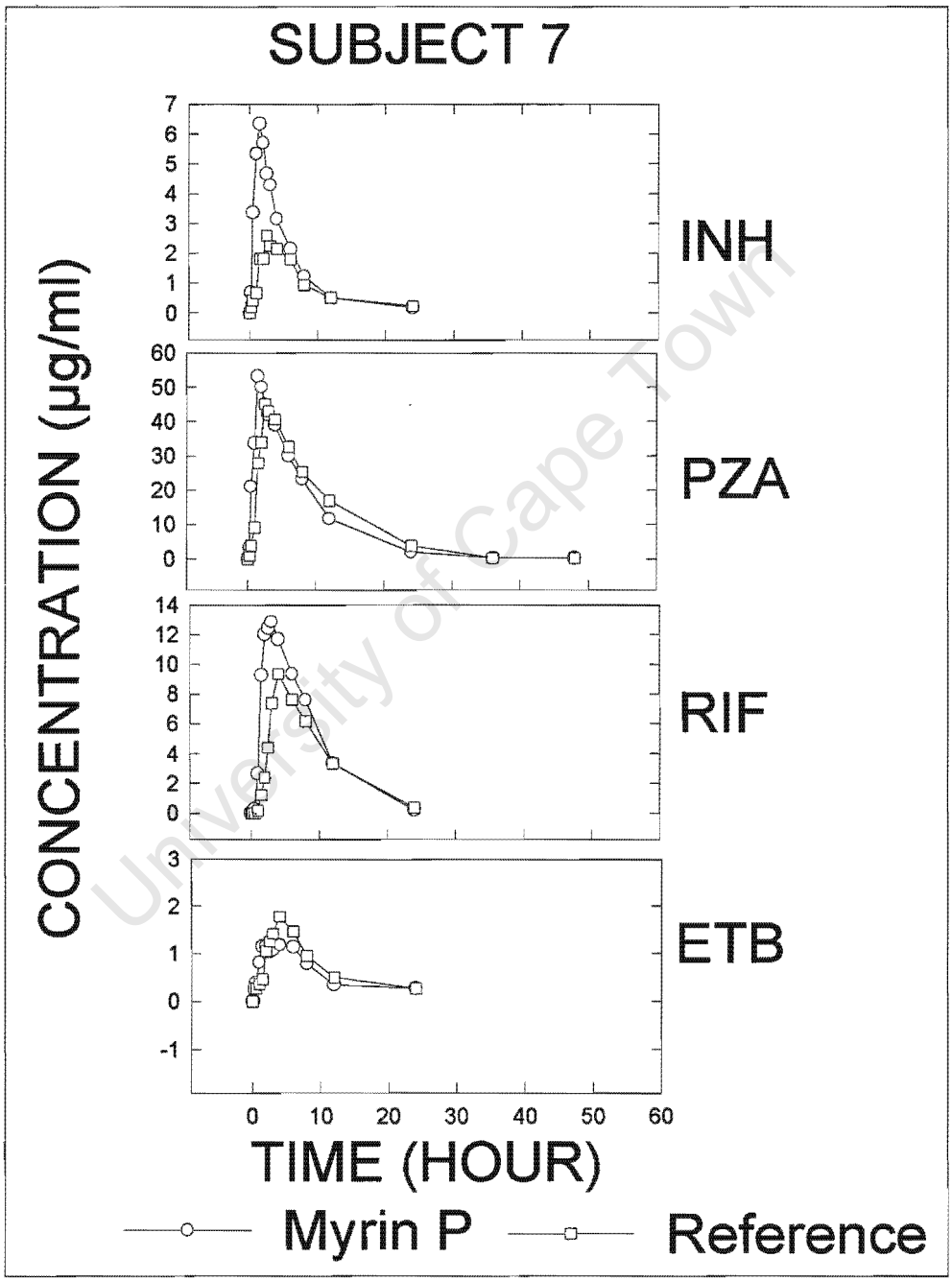
The files are named as follows in order of the respective studies with the dat file extention.

Study1.dat
Study2.dat
Study3.dat
Study4.dat
Study5.dat
Study6.dat
Study7.dat
Study8.dat
Study9.dat
Study10.dat
Study11.dat
Study12.dat
Study13.dat

Appendix 3

Pharmacokinetic profile

A selected pharmacokinetic profile as determined from concentration –time curve plots indicating the subject number in study, drugs present in the combination and the test and reference graphs respectively.



Appendix 4

Randomization sequence of studies investigated

Study	Reference/Test
Study 1 (Period 2)	1, 2, 3, 4, 5, 6,7, 8, 9, 10, 11, 12, 13, 14, 15, 16
150/300	2, 6, 7, 8, 9, 11, 12, 15
300/150	1, 3, 4, 5, 10, 13, 14, 16
Study 2	1, 3, 5, 6, 7, 10, 14, 16, 17, 18, 23, 24
	2, 4, 8, 9, 11, 12, 13, 15, 19, 20, 21, 22
Study 3	1, 3, 9, 19, 20, 22
	2, 7, 10, 11, 13, 14, 15, 16, 17, 21
Study 4	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
	13, 14, 15, 16, 17, 18, 19, 20, 21, 22
Study 5	1, 4, 8, 9, 10, 12, 13, 16, 18
	2, 3, 5, 6, 7, 14, 17, 19, 21, 22
Study 6	1, 3, 6, 8, 10, 11, 18, 19, 20, 21, 22, 24
	2, 4, 5, 7, 9, 12, 13, 14, 15, 16, 17, 23
Study 7	1, 2, 5, 6, 7, 8, 11, 15, 19, 20, 23, 24
	3, 4, 9, 10, 12, 13, 16, 17, 18, 21, 22
Study 8	2, 3, 5, 6, 9, 14, 15, 16, 17, 19
	1, 4, 7, 8, 10, 12, 13, 18, 20
Study 9	3, 4, 5, 6, 10, 12, 13, 14, 16, 19, 21, 22
	1, 2, 7, 8, 9, 11, 15, 17, 18, 20, 23, 24
Study 10	2, 4, 9, 11, 12, 14, 15, 16, 19, 22, 23
	1, 3, 5, 6, 7, 8, 10, 13, 17, 18
Study 11	2, 5, 8, 10, 13, 15, 17, 18, 21, 23
	1, 4 , 6, 7, 9, 11, 12, 16, 19, 20, 22, 24
Study 12	1, 2, 4, 5, 8, 10, 14, 17, 18, 21
	3, 6, 7, 9, 11, 15, 16, 19, 20, 22, 23, 24
Study 13	1, 2, 7, 8, 9, 11, 12, 14, 18, 21, 23, 24
	3, 4, 5, 6, 10, 13, 15, 16, 17, 19, 20, 22

Appendix 5
Publications

McIlleron, H., **Gabriels, G.**, Smith, P. , E., Fourie, P.B. Ellard, G. (1999) The development of a standardised screening protocol for the in vivo assessment of rifampicin bioavailability Int. J. Tuberc Lung Dis, 3(11): S329-S335.

Pillai, G., Fourie, P.B., Padayatchi, N., Onyebujoh, P.C., McIlleron, H., Smith, P., **Gabriels, G.** (1999). Recent bioequivalence studies on fixed-dose combination anti-tuberculosis drug formulations available on the global market. Int. J. Tuberc Lung Dis, 3(11): S309-S316.

INT J TUBERC LUNG DIS 3(11):S329–S335

The development of a standardised screening protocol for the in vivo assessment of rifampicin bioavailability

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SUMMARY

SETTING: The prerequisite for in vivo bioavailability testing of rifampicin in fixed-dose combination (FDC) formulations is widely accepted. However, many smaller drug regulatory authorities and drug manufacturers have difficulty implementing costly and cumbersome testing procedures.

OBJECTIVE: To test whether a simplified blood sampling schedule can be used for the determination of drug bioequivalence in randomised, single dose, crossover studies of FDCs and appropriate reference formulations.

METHOD: The results of three bioavailability and bioequivalence studies of different rifampicin-containing FDCs were analysed. The relationship between the number of time points employed and precision of estimated relative bioavailability was explored. The relative bioavailabilities of the drug components in the test FDCs were calculated using maximal concentration and area under the curve estimates based on an extended blood sampling schedule of up to 15 time points over 48 hours, and a contracted sampling scheme with only six blood samples over 8 hours.

RESULTS: Estimates of relative bioavailability calculated using the contracted blood sampling protocol were closely similar to those derived using the extended sampling schedules.

CONCLUSION: Considerable cost and convenience benefits can be gained by using the contracted sampling schedule with only a minor reduction in the precision of the estimation of relative rifampicin bioavailability.

KEY WORDS: tuberculosis; fixed-dose combination; bioavailability

THE NEED TO ESTABLISH adequate bioavailability of rifampicin in fixed-dose combinations (FDCs) has long been recognised. Acocella highlighted the importance of demonstrating the quality of rifampicin-containing FDCs at the annual meeting of the International Union Against Tuberculosis and Lung Disease (IUATLD) held in Dubrovnik in 1988. In a symposium entitled 'The quality of antituberculosis drugs' he presented the findings of an investigation into the bioavailability of formulations containing rifampicin alone, rifampicin and isoniazid, and three-drug FDCs containing rifampicin, isoniazid and pyrazinamide. He demonstrated that one of three two-drug combinations and all of four three-drug combinations had grossly inferior bioavailability for rifampicin compared to reference single-drug products.[1] Acocella concluded that the concentrations of rifampicin reached at the site of infection after dosing with these agents were likely to be ineffective due to the dose dependency of the antituberculosis activity of rifampicin, and that the formulations were therefore not suitable for clinical use.[1]

By contrast, the results of Ellard et al.'s crossover study in patients in 1986 demonstrated excellent bioavailability of rifampicin, isoniazid and pyrazinamide in two three-drug fixed-dose Rifater combinations.[2] In addition to determination of the plasma levels of rifampicin, isoniazid and pyrazinamide at 1, 2, 4, 6, 9 and 24 hours, urinary excretion of the three drugs and their principal metabolites was measured to 48 hours. Both sets of measurements confirmed that the bioavailability of the FDCs was similar to that of the single-drug reference formulations. The urinary findings also lent support to the conclusions of Brechbühler et al.[3] that the urinary excretion of rifampicin plus desacetyl-rifampicin can be used to measure bioavailability.

Subsequent to the 1988 meeting authorities advised against the use of triple-drug FDCs unless they were of reputable origin and had unquestionably demonstrated bioavailability.[4,5] The administration of drugs in FDCs holds major advantages over that of several single-drug preparations:[6] simplified prescribing, dispensing and administration, and the ease of swallowing fewer tablets, results in more effective delivery of

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Table 1 Dates and drug dosages for each of the studies

Study	Date	Dosage of test formulations and the reference agents (mg)			
		Rifampicin	Isoniazid	Pyrazinamide	Ethambutol
1: test formulation 1	10/1996	600	300	1500	1125
2: test formulation 2	10/1997	600	300	1500	1000
3: test formulation 3	02/1998	600	250	1500	0

the treatment to patients. It follows that an efficient and standardised method is needed to test the quality of an FDC before it is implemented in treatment programmes.

In vivo demonstration of adequate absorption from FDCs is especially important for rifampicin. In addition to the evidence of bioavailability problems resulting from variations in particle size, crystalline form, excipients and the manufacturing process,[7] the drug has low solubility in water and hence potential absorption problems, and is subject to enterohepatic circulation,[8] which could complicate bioavailability assessments.

The World Health Organization (WHO) and the IUATLD are engaged in a collaborative initiative to assist small National Drug Regulatory Authorities to ensure acceptable standards of quality, safety and efficacy of FDCs used or manufactured under their jurisdiction, including the promotion of a technical basis for assuring interchangeability of multi-source products. Simultaneous with the initiation of this venture, the Department of Pharmacology at the University of Cape Town (UCT), South Africa, has been conducting a number of bioavailability studies of FDCs since 1996, largely for registration purposes, and according to guidelines issued by the WHO.[9]

In response to the need for a standardised screening protocol to establish a minimum standard for the conduct of bioequivalence studies of rifampicin-containing FDCs, the Department of Pharmacology, UCT, has collaborated with the WHO. Analysis of the studies of three FDCs has provided evidence that a contracted and more efficient protocol can be used without significantly compromising the precision of the rifampicin bioavailability estimations.

METHODS

The three studies analysed were selected from the nine conducted in the department since September 1996, as they would provide comparative data on estimating the relative bioavailabilities of three of the key antituberculosis drugs, and because of the previous evidence[1] that manufacturing FDCs with acceptable rifampicin bioavailability was likely to be most demanding when three or more drugs were included. The formulations were of solid/tablet form and were found to be fully bioavailable and bioequivalent to the single-drug reference agents. Formulations 1 and 2 were recently developed products and the studies were conducted for registration purposes. Formulation 3 was a generic formulation manufactured in Malta for the International Dispensary Association.

Two of the formulations were four-drug FDCs of rifampicin, isoniazid, pyrazinamide and ethambutol. For test formulation 1 each tablet contained rifampicin 120 mg, isoniazid 60 mg, pyrazinamide 300 mg, and ethambutol 225 mg; for test formulation 2 each tablet contained rifampicin 120 mg, isoniazid 60 mg, pyrazinamide 300 mg and ethambutol 200 mg; the third FDC (test formulation 3) was a three-drug combination containing rifampicin 120 mg, isoniazid 50 mg and pyrazinamide 300 mg per tablet. The total drug doses used in each study are given in Table 1.

A crossover design was used for each study, in which each subject was randomised to receive a single dose of either the test formulation or the reference agents at the first drug administration (Unpublished study reports for MyrinP [1996] and Rifafour [1997] FDCs).[10] Twenty-two to 24 healthy volunteers of either sex, aged between 18 and 55 years and who weighed more than 50 kg were enrolled (the aim was

Table 2 Peak rifampicin plasma concentrations (C_{max}), time to reach C_{max} and AUC_t for the three studies. For studies 1 and 2 the area under the curve was calculated to 24 hours, while that for study 3 was calculated to 12 hours. Standard errors are displayed in parentheses.

Study	No. of subjects	C_{max} (μg/ml)		T_{max} (hours)		AUC_t		AUC_{0-12}	
		Test	Reference	Test	Reference	Test	Reference	Test	Reference
1	24	12.9 (0.6)	13.0 (0.9)	2.2 (0.1)	1.7 (0.1)	74.5 (4.5)	70.7 (4.7)	65.7 (3.3)	62.4 (3.7)
2	23	10.5 (0.5)	10.1 (0.6)	2.2 (0.1)	2.3 (0.1)	66.7 (3.2)	60.5 (3.5)	58.2 (2.5)	52.4 (3.0)
3	19	12.3 (0.8)	12.2 (0.8)	1.6 (0.2)	2.0 (0.1)	64.9 (4.8)	64.3 (5.4)	64.9 (4.8)	64.3 (5.4)

Table 3 Peak isoniazid plasma concentrations (C_{max}), time to reach C_{max} (T_{max}) and AUC_{0-24} for two studies. Standard errors are expressed in parentheses.

		C_{max} (μg/ml)		T_{max} (hours)		AUC_{0-24}	
Study	No. of subjects	Test formulation	Reference	Test formulation	Reference	Test formulation	Reference
1	24	3.5 (0.2)	3.6 (0.2)	1.6 (0.2)	1.2 (0.1)	20.2 (1.4)	18.5 (1.3)
2	23	4.4 (0.4)	4.3 (0.4)	1.1 (0.2)	1.1 (0.2)	22.8 (1.9)	23.4 (2.1)

to ensure that 20 completed each study). Smokers and volunteers with a history of drug or alcohol abuse were excluded, as were those with a history of drug allergy, severe asthma or active or recurrent allergic disease. No volunteer previously treated for tuberculosis or with liver disease, renal disease or gastrointestinal disorder was included. Screening laboratory evaluations of liver and renal function and haematological profiles were normal. Written informed consent was obtained from each subject prior to study admission.

All subjects were informed of possible side-effects of the drugs and that they were free to withdraw from the study at any stage without prejudice to him/her for doing so. The studies were approved by the University of Cape Town's Research Ethics Committee.

Subjects were required to refrain from alcohol consumption and strenuous exercise for 48 hours before and after drug administration. No drug or medication that might alter drug metabolism or elimination was permitted in the month prior to study initiation, or during the study.

The FDC formulations (test formulations 1, 2 and 3 for studies 1, 2 and 3, respectively) were compared to equivalent amounts of internationally accepted and registered single-drug products (reference agents). The dose of rifampicin at each drug administration was 600 mg, and the doses of isoniazid, pyrazinamide and ethambutol were also in keeping with the recommended therapeutic range for adults weighing 50 kg or more.[11] The drugs were administered as a single dose with 200 ml of water, with a washout period of one week between the drug administrations.

Subjects fasted for 8 hours before drug administration. Three hours after drug administration a light standardised breakfast was provided. Five hours following drug administration, free fluid intake was permitted.

Adverse events were monitored clinically and followed to resolution. If withdrawal of a subject became necessary after the first drug administration the subject was not replaced and the pharmacokinetic data following the first drug administration were excluded from the analysis.

Venous blood specimens were collected by venepuncture for high performance liquid chromatography (HPLC) analysis of drug concentrations. Blood samples were collected into two lithium-heparin coated glass tubes at approximately 0.5 hours prior to drug administration and at 0.25, 0.5, 1,1.5, 2, 2.5, 3, 4,6, 8,12, 24, 36 and 48 hours after drug administration for studies 1 and 2. The samples were analysed for rifampicin, isoniazid, pyrazinamide and ethambutol levels, except those taken at 36 and 48 hours, which were analysed for pyrazinamide only. For study 3, the blood sampling schedule differed only in that the 0.25 hour sample was omitted, an additional sample was collected at 10 hours, and the last blood sample was at 12 hours. Blood samples from study 3 were analysed for rifampicin concentrations only.

The samples were stored on ice for a maximum of 1 hour, until centrifugation, transfer of approximately 1.2 ml of plasma into each of two dry polypropylene tubes and storage at −80°C. The rifampicin, isoniazid and pyrazinamide assays were performed by a validated HPLC method.[12,13] The inter-day and intra-day coefficient of variation for the assays was <6%, while the limit of reliable detection for rifampicin, isoniazid and pyrazinamide was 0.05 μg/ml. Plasma concen-

Table 4 Estimates of relative isoniazid bioavailability of formulation 2 (as percentages) by the procedure of Hauschke et al.[17] for study 2

Parameter	Median/point estimate	90% confidence interval	% span
C_{max} contracted	110.5	98.0–125.2	27.2
C_{max} full data	101.5	90.0–111.6	21.6
AUC_{0-8} contracted	101.5	94.7–110.5	15.9
AUC_{0-8} all data	97.0	89.1–105.1	16.0
AUC_{0-12} all data	98.0	90.9–106.7	15.8
AUC_{0-24} all data	100.1	91.4–113.9	22.5

Table 5 Estimates of relative ethambutol bioavailability of formulation 1 (as percentages) by the procedure of Hauschke et al.[17]

Parameter	Median/point estimate	90% confidence interval	% span
C_{max} contracted	87.4	68.9–110.5	41.6
C_{max} full data	97.1	84.3–114.7	30.4
AUC_{0-8} contracted	109.0	86.1–136.5	50.4
AUC_{0-8} all data	114.7	94.2–134.5	40.3
AUC_{0-12} all data	110.5	94.4–133.2	38.8
AUC_{0-24} all data	109.7	92.5–138.8	46.3

Table 6 Estimates of relative rifampicin bioavailability of test formulation 1 (as percentages) by the procedure of Hauschke et al.[17]

Parameter	Median/point estimate	90% confidence interval	% span
C_{max} contracted	89.5	81.3–100.3	19.0
C_{max} full data	104.2	95.3–114.1	18.8
AUC_{0-8} contracted	99.8	93.3–107.4	14.1
AUC_{0-8} all data	103.4	96.8–111.4	14.6
AUC_{0-12} all data	106.3	100.0–114.9	14.9
AUC_{0-24} all data	106.1	99.0–114.3	15.3

Table 8 Estimates of relative rifampicin bioavailability of test formulation 3 (as percentages) by the procedure of Hauschke et al.[17]

Parameter	Median/point estimate	90% confidence interval	% span
C_{max} contracted	104.5	90.7–119.8	29.1
C_{max} full data	100.5	89.2–115.2	26.0
AUC_{0-8} contracted	100.7	91.5–113.8	22.3
AUC_{0-8} all data	102.2	91.7–115.5	23.8
AUC_{0-12} all data	101.2	91.9–112.4	20.5

trations of ethambutol were also determined by HPLC using a modification of the gas liquid chromatography (GLC) method of Lee and Wang,[14] with a analytical detection limit of 0.1 μg/ml.

Concentration-time curves were plotted for each series of drug assays using both the full set of sampling times (as described above) and a contracted set of sampling times, where the concentrations determined at 1, 2, 4, 6 and 8 hours only were used. For each concentration-time curve, maximum drug concentration (C_{max}) was taken as the highest drug level measured, and the areas under the curve $(AUC)_{0-8}$, AUC_{0-12}, and AUC_{0-24} (or AUC_{0-48} for pyrazinamide) denoting the areas under the curve to 8, 12, 24 or 48 hours, respectively, were calculated using the trapezoidal rule. AUC_{0-8} was calculated using the data from all the sampling times between 0 and 8 hours, and by using the concentrations determined at 0, 1, 2, 4, 6 and 8 hours only.

Bioequivalence analysis of C_{max}, AUC_{0-8}, AUC_{0-12} and AUC_{0-24} (or AUC_{0-48} for pyrazinamide), and C_{max} and AUC_{0-8} were conducted for the full and contracted sets of sampling times respectively. The statistical method described by Hauschke et al.[15] was used to determine the 90% confidence interval (90% CI) for the test/reference ratio for each set of variables. For bioequivalence the 90% CI should lie within the range 80–125%.

RESULTS

The pharmacokinetic parameters of rifampicin, isoniazid, pyrazinamide and ethambutol determined for those measures of principle interest are shown in Tables 2, 3, 12 and 10, respectively. Detectable amounts of rifampicin, isoniazid, pyrazinamide or ethambutol were not found in any of the pre-treatment samples. By 24 hours rifampicin concentrations had fallen to below detectable limits. Rifampicin was rapidly absorbed from all three test formulations; the results suggest that bioavailability is likely to be excellent (Table 2).

The bioequivalence analyses for rifampicin are shown in Tables 6, 7 and 8. The similarity between the results for the AUC to 12 hours and that to 24 hours can be attributed to the negligible 24-hour rifampicin levels. More precise estimates of relative bioavailability were obtained using AUCs as compared with C_{max}-based measures. Furthermore, the AUC_{0-8}s calculated using the contracted time points were closely similar to the AUCs calculated using more expanded sampling times, the 90% CI spans increasing on average by only about 1% using all the data points.

These studies thus all showed unequivocally that rifampicin was excellently absorbed from all three

Table 7 Estimates of relative rifampicin bioavailability of test formulation 2 (as percentages) by the procedure of Hauschke et al.[17]

Parameter	Median/point estimate	90% confidence interval	% span
C_{max} contracted	103.5	93.7–118.8	25.1
C_{max} full data	101.8	92.9–119.1	26.2
AUC_{0-8} contracted	110.2	101.2–125.0	23.8
AUC_{0-8} all data	116.5	105.6–130.1	24.5
AUC_{0-12} all data	109.8	101.4–122.9	21.5
AUC_{0-24} all data	108.3	100.4–120.5	20.1

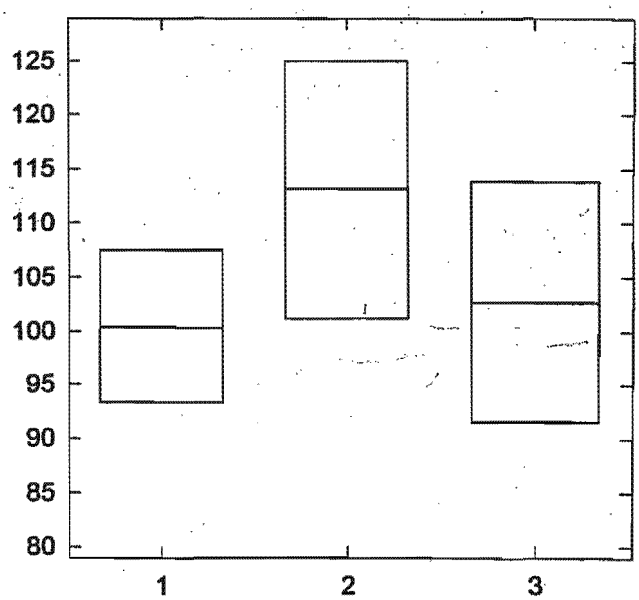

Figure 90% confidence intervals of the test/reference ratio of the AUC_{0-8} calculated using contracted sampling times for each of the three studies.

Table 9 Estimates of relative isoniazid bioavailability of formulation 1 (as percentages) by the procedure of Hauschke et al.[17] for study 1.

Parameter	Median/point estimate	90% confidence interval	% span
C_{max} contracted	97.2	88.9–112.5	23.6
C_{max} full data	92.9	80.1–104.9	24.8
AUC_{0-8} contracted	110.6	101.3–120.2	18.9
AUC_{0-8} all data	107.3	100.3–116.9	16.6
AUC_{0-12} all data	110.1	104.0–117.4	13.4
AUC_{0-24} all data	113.9	107.6–122.2	14.6

Table 11 Estimates of relative ethambutol bioavailability of formulation 2 (as percentages) by the procedure of Hauschke et al.[17]

Parameter	Median/point estimate	90% confidence interval	% span
C_{max} contracted	110.5	98.0–125.2	27.2
C_{max} full data	101.5	90.0–111.6	21.6
AUC_{0-8} contracted	101.5	94.7–110.5	15.9
AUC_{0-8} all data	97.0	89.1–105.1	16.0
AUC_{0-12} all data	98.0	90.9–106.7	15.8
AUC_{0-24} all data	100.1	91.4–113.9	22.5

test FDCs, which were essentially bioequivalent (Figure). They also demonstrate apparently significant variability in the spans of the 90% CI; other evidence suggests that this was almost certainly due to individual variability in the handling of rifampicin by the volunteers rather than differences in analytical precision between the studies.

Isoniazid showed rapid and excellent absorption, as demonstrated in Table 3. Considerably more precise estimates of relative bioavailability were achieved using AUCs than C_{max}s (Tables 4 and 9).

As demonstrated in Tables 5, 10 and 11, ethambutol in both test formulations was excellently absorbed, and although wide spans were observed for the 90% bioavailability confidence intervals, the AUCs calculated using more expanded sampling schedules resulted in only minimal gains in the precision of the relative bioavailability estimates (Tables 5 and 11).

Pyrazinamide was also rapidly and excellently absorbed (Table 12). The 90% CI for AUC_{0-8} using the contracted sampling schedule correlated well with the AUCs calculated from all data; and the C_{max} calculated from the contracted sampling times is also similar to that obtained using the expanded schedule (Tables 13 and 14). Although the AUCs give slightly smaller spans than the C_{max} values, the ranges are so tight (because of the rapid absorption of this drug which is both highly water soluble and also penetrates body membranes extremely efficiently), even with C_{max} data, the chance of their breaching the 80–125% 90% CI range is most unlikely.

Hence the bioequivalence of all four drugs, rifampicin, isoniazid, ethambutol and pyrazinamide can be assessed using the contracted sampling schedule only, over an 8-hour period. The method is convenient, obviates the need for hospitalisation of subjects, requires considerably fewer analyses, and as a consequence is considerably cheaper than the methods used previously.

DISCUSSION

It is currently recommended that the bioavailability of rifampicin in fixed-dose combinations be demonstrated to compare favourably with that of suitable single-drug reference formulations by in vivo studies prior to registration or licensing with national authorities. The 90% confidence intervals of the bioequivalence of rifampicin in fixed-dose combinations should lie between 80% and 125% before registration.[9] In addition, it may be advisable to include in vivo demonstration of the continuing bioavailability of rifampicin in the assessment of the quality of further batches of licensed FDC formulations, especially if there is reason to suspect that an excipient or manufacturing process might have been changed.[4,16]

Simplification of registration requirements, and minimum standards for licensing and ongoing quality assurance for FDCs, is desirable, as they hold definite advantages over the administration of several single-drug formulations. A more efficient protocol that retains the precision of more extended procedures used previously should encourage the more widespread regulation of rifampicin-containing FDCs.

The analysis of the three studies as described above supports the use of the restricted 0-8 hour sampling scheme for the demonstration of bioequivalence for rifampicin in FDC anti-tuberculosis drug formulations.[6,10]

Proving bioequivalence of isoniazid, ethambutol

Table 10 Peak ethambutol plasma concentrations (C_{max}), time to reach C_{max} (T_{max}) and AUC_{0-24} for two studies. Standard errors are shown in parentheses.

Study	No. of subjects	C_{max} (μg/ml)		T_{max} (hours)		AUC_{0-24}	
		Test	Reference	Test	Reference	Test	Reference
1	24	3.3 (0.2)	3.4 (0.2)	2.7 (0.1)	2.6 (0.1)	13.6 (0.7)	13.1 (1.0)
2	23	3.7 (0.3)	3.5 (0.2)	2.8 (0.2)	2.9 (0.1)	15.8 (1.4)	14.9 (0.8)

Table 12 Peak pyrazinamide plasma concentrations (C_{max}), time to reach C_{max} (T_{max}) and AUC_{0-48} for two studies. Standard errors are shown in parentheses.

Study	No. of subjects	C_{max} (μg/ml)		T_{max} (hours)		AUC_{0-48}	
		Test	Reference	Test	Reference	Test	Reference
1	24	32.3 (1.0)	33.8 (0.9)	1.8 (0.2)	1.7 (0.2)	538 (19)	520 (16)
2	23	42.6 (2.2)	42.5 (2.6)	1.3 (0.2)	1.4 (0.2)	598 (32)	591 (23)

and pyrazinamide in FDCs is less exacting, and in most instances dissolution testing is adequate. However, parallel HPLC analyses of plasma isoniazid, pyrazinamide and ethambutol concentrations, as was done in this study, was achieved with only a modest increase in costs over that for estimating rifampicin alone. The bioequivalence confidence interval analysis demonstrated that in vivo bioavailability of isoniazid, pyrazinamide and ethambutol can be assessed using the contracted sampling schedule to 8 hours only.

Determination of the urinary excretion of rifampicin and desacetyl-rifampicin represents another way in which rifampicin bioavailability assessment might be simplified, an approach which is currently being investigated by the authors.

The methods described in this paper are designed to assess the average bioequivalence of FDC formulations for regulatory purposes and thus prevent the release of substandard preparations. The methods focus on the comparison of the population averages between the test and reference products, thus addressing the question of 'prescribability'. Broadly, the concept of bioequivalence also encompasses aspects of interchangeability of importance for 'switchability', or individual bioequivalence,[17] a topic that is not addressed in this paper. Clear guidelines on methods to assess these controversial aspects for regulatory purposes have yet to be promulgated.

Limitations of the study include the retrospective analysis of three studies from a single centre. The approach should therefore be further validated to eliminate any bias that may have occurred.

Table 13 Estimates of relative pyrazinamide bioavailability of formulation 1 (as percentages) by the procedure of Hauschke et al.[17]

Parameter	Median/point estimate	90% confidence interval	% span
C_{max} contracted	100.4	95.5–107.1	11.6
C_{max} full data	95.9	91.1–100.2	9.1
AUC_{0-8} contracted	102.0	98.7–107.3	8.6
AUC_{0-8} all data	99.8	97.3–104.0	6.7
AUC_{0-48} all data	105.8	103.1–108.6	5.5

Table 14 Estimates of relative pyrazinamide bioavailability of formulation 2 (as percentages) by the procedure of Hauschke et al.[17]

Parameter	Median/point estimate	90% confidence interval	% span
C_{max} contracted	102.0	98.5–105.1	6.6
C_{max} full data	104.1	100.5–111.1	10.6
AUC_{0-8} contracted	99.5	96.6–102.5	6.0
AUC_{0-8} all data	98.0	94.7–101.5	6.9
AUC_{0-12} all data	98.0	95.1–101.5	6.4
AUC_{0-42} all data	99.0	95.6–103.1	7.5

CONCLUSION

A comparison of the results of the statistical analysis for bioequivalence applied to both the extended and the contracted sampling times indicates that little benefit is gained from the application of a more extended sampling schedule, and that fewer sampling times can be used without significantly compromising the precision of rifampicin bioavailability estimations. Although the three studies analysed were selected from a single centre on a retrospective basis, the results of this analysis strongly support the use of the WHO/IUATLD-recommended protocol for establishing the rifampicin bioequivalence of FDCs.[10,18] The use of this method, in which only six blood samples are required up to 8 hours after drug administration, is considerably more convenient and economical, and will hopefully lead to more widespread control of the quality of antituberculosis FDCs in developing nations.

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Recent bioequivalence studies on fixed-dose combination anti-tuberculosis drug formulations available on the global market

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SUMMARY

SETTING: Concern has been expressed about the bioavailability of rifampicin in some fixed-dose combination (FDC) anti-tuberculosis formulations.

OBJECTIVE: To evaluate the relative bioavailability of rifampicin in various FDC formulations currently in use in tuberculosis control programmes in the global market.

DESIGN: A two-period randomised crossover bioequivalence study in healthy male volunteers, with a 1 week washout period between treatments. Plasma rifampicin concentrations were measured at 0, 1, 2, 4, 6, 8 and 12 hours after each drug administration.

RESULTS: The AUC_{0-8}, AUC_{0-12} and C_{max} for rifampicin in seven of 10 FDC formulations was not found to be bioequivalent to the reference administered as loose (separate) formulations. This was confirmed using parametric and non-parametric statistical methods.

CONCLUSIONS: The poor relative bioavailability of rifampicin from some FDCs has been documented. The implications for tuberculosis programmes are extremely serious and warrant urgent attention.

KEY WORDS: bioavailability; fixed-dose combination; rifampicin; tuberculosis

IN THE TREATMENT of tuberculosis, drugs formulated in fixed-dose combinations might hold several advantages over administration of the individual drugs separately. These include the following:[1–4] prescribing is simpler; physicians' prescribing errors are minimised; patients are less likely to be confused about numbers of tablets and capsules to be taken in self administered dosage schedules; drug doses can be more precisely adjusted to the patient's weight; and the number of pills to be swallowed is reduced.

The main advantage, however, is a public health issue. Since the drugs are taken in combination, the possibility of patients only taking a single drug is prevented and hence the risk of failure during treatment, as a result of the selection of drug-resistant strains, is greatly reduced. This is based on the assured ingestion of rifampicin and isoniazid—the important 'resistance preventing agents'[5] in the tuberculosis treatment regimen.

However, concern has been expressed about the apparent inadequate bioavailability of rifampicin in some fixed-dose combination (FDC) preparations.[6–8] Consequently, international regulatory authorities recommend that the bioavailability of formulations be compared with that of preparations of reputable efficacy. Only those shown to be bioequivalent should be released onto the market.[7,9]

This paper represents part of a comprehensive project in which the quality of FDC preparations currently in use in various TB control programmes in the global market will be assessed. We report here on the rifampicin bioavailability from 10 FDCs: three containing rifampicin and isoniazid only (two-drug combinations), five containing rifampicin, isoniazid and pyrazinamide (three-drug combinations) and two containing rifampicin, isoniazid, pyrazinamide and ethambutol (four-drug combinations).

METHODS

Acquisition of study medications

The FDCs under investigation (Table 1) were sourced in terms of the market representation of the manufacturers.[10] Representatives of the World Health Organization (WHO) and the International Union Against Tuberculosis and Lung Disease (IUATLD) purchased the medications from wholesale/retail outlets rather than from the manufacturer. These were supplied to the South African Medical Research Council (MRC), where they were repackaged into airtight plastic tubes, coded and labelled with the content of active ingredients. Repackaging was necessary in order to ensure that the clinical investigators were blinded to the identity (brand name) or country of origin of

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Table 1 Anti-tuberculosis fixed-dose combination (FDC) formulations under investigation and reference formulations used in the bioequivalence studies

FDC formulations Study code	Generic name	Amount per tablet/capsule	Number administered	Total dose (mg)
Formulation N	Rifampicin	120	5 tablets	600
	Isoniazid	50		250
	Pyrazinamide	300		1500
Formulation C	Rifampicin	225	2 tablets	450
	Isoniazid	150		300
	Pyrazinamide	500		1000
Formulation E	Rifampicin	225	2 tablets	450
	Isoniazid	150		300
	Pyrazinamide	750		1500
	Ethambutol	400		800
Formulation H	Rifampicin	150	4 tablets	600
	Isoniazid	100		400
	Pyrazinamide	500		2000
	Ethambutol	267		1068
Formulation P	Rifampicin	300	2 capsules	600
	Isoniazid	150		300
Formulation D	Rifampicin	150	4 capsules	600
	Isoniazid	100		400
Formulation J	Rifampicin	225	2 capsules	450
	Isoniazid	150		300
	Pyrazinamide	750		1500
Formulation M	Rifampicin	120	5 tablets	600
	Isoniazid	80		400
	Pyrazinamide	250		1250
Formulation Q	Rifampicin	150	4 capsules	600
	Isoniazid	100		400
Formulation F	Rifampicin	225	2 tablets	450
	Isoniazid	150		300
	Pyrazinamide	750		1500

Reference formulations

Trade name	Generic name	Manufacturer	Amount per tablet (mg)
Rificin 600 mg	Rifampicin	Rolab (Pty) Ltd	600
Rifcin 450 mg	Rifampicin	Rolab (Pty) Ltd	450
Be-Tabs Isoniazid 100	Isoniazid	Be-Tabs Pharmaceuticals (Pty) Ltd	100
Pyrazide	Pyrazinamide	Noristan Ltd	500
Purderal P 400 mg tablets	Ethambutol	Pharmacare Ltd	400

the formulations. All formulations were then stored protected from light at a temperature of approximately 4°C and in a limited-access area.

Preference for screening was in the order of four-drug, three-drug and two-drug combinations, randomised internally per group.

Bioequivalence studies

A two-period randomised cross-over study design was conducted, utilising at least 18 healthy male volunteers. Strict inclusion and exclusion criteria were applied.[11] Drugs were administered as a single bolus with 200 ml of tap water in therapeutic doses after an overnight fast.

Blood samples were collected into heparinised tubes at 0, 1, 2, 4, 6, 8 and 12 hours after each drug administration using an in-dwelling venous cannula. Urine samples were collected from 0–4 hours and from 4–8 hours (data not presented in this manuscript).

Three hours following drug administration, a light standardised breakfast was served. Lunch was served 5 hours after drug administration, and thereafter fluid was allowed ad libitum.

After a one week drug-free washout period, volunteers received the alternative study medications in exactly the same dosages (Table 1).

Drug assay methodology

Plasma rifampicin concentrations were determined using a validated high performance liquid chromatographic (HPLC) assay technique at the Department of Pharmacology, University of Cape Town. The assay procedure was developed in-house.[12]

Quantitation of the chromatograms used the peak area method. The inter-day and intra-day coefficient of variation for the assays was <6%, while the limit of quantitation (LQ) for rifampicin was 0.05 μg/ml.[13]

Statistical and kinetic analysis of bioequivalence data

The data from the bioequivalence studies were subjected to non-compartmental analysis. Concentration-time curves were plotted for each series of drug assays. From these plots, C_{max} was taken as the highest drug concentration measured.

The area under the plasma concentration-time curve until 8 hours and until the last time measured, i.e., 12 hours (AUC_{0-8} and AUC_{0-12} for rifampicin), was determined by the (linear/logarithmic) trapezoidal rule.

These parameters were subjected to analysis of variance (ANOVA) after log transformation of the data and using formulation, subject, sequence and period as the treatment effects.

90% confidence intervals (90% CI) were computed for the ratio of test/reference using both parametetic methods (based on the ANOVA) and non-parametric methods.[14] Bioequivalence was declared if the 90% CI for the test/reference ratio lay completely in the range 80-125%. In addition, for the parametric analysis the null hypotheses for both the two one-sided *t* tests[15] had to be rejected at the $P < 0.05$ level before bioequivalence could be declared.

Statistical analyses were conducted using WinNonlin Pro Version 1.5 (Scientific Consulting Inc.) and Microsoft Excel 97.

RESULTS

The composition of the 10 rifampicin-containing FDCs and that of the reference formulations is recorded in Table 1. There were three two-drug combinations (formulations P, D and Q), five three-drug combinations (formulations N, C, J, M and F), and two four-drug combinations (formulations E and H).

The rifampicin concentration versus time profiles for the 10 formulations relative to the reference formulation are illustrated in Figures 1–10. Formulations N, D and J were the only three formulations that were found to be bioequivalent (Table 2).

All of the seven formulations that were not found to be bioequivalent to the reference formulation displayed 90% CIs that included values that were below 80%. In the case of formulations C and F, all 90% CIs were below 80%.

There was very good agreement between the parametric and non-parametric statistical analyses conducted on this data. There was also good agreement between the parameters of AUC_{0-8} and AUC_{0-12}.

DISCUSSION AND CONCLUSIONS

Seven of the 10 rifampicin-containing FDC formulations investigated in this study were *not* found to be bioequivalent according to internationally accepted criteria for bioequivalence. This raises cause for concern that these formulations are currently in use in tuberculosis control programmes.

It is clear, therefore, that the problem of poor bioavailability of rifampicin from FDCs, first revealed by Acocella[6] and suspected in India by Fox,[8] is still serious. However, although the probability of poor bioavailability from FDCs has been suggested over the past decade, this is the first study to comprehensively document the problem using a post-marketing surveillance strategy and a randomised cross-over study design.

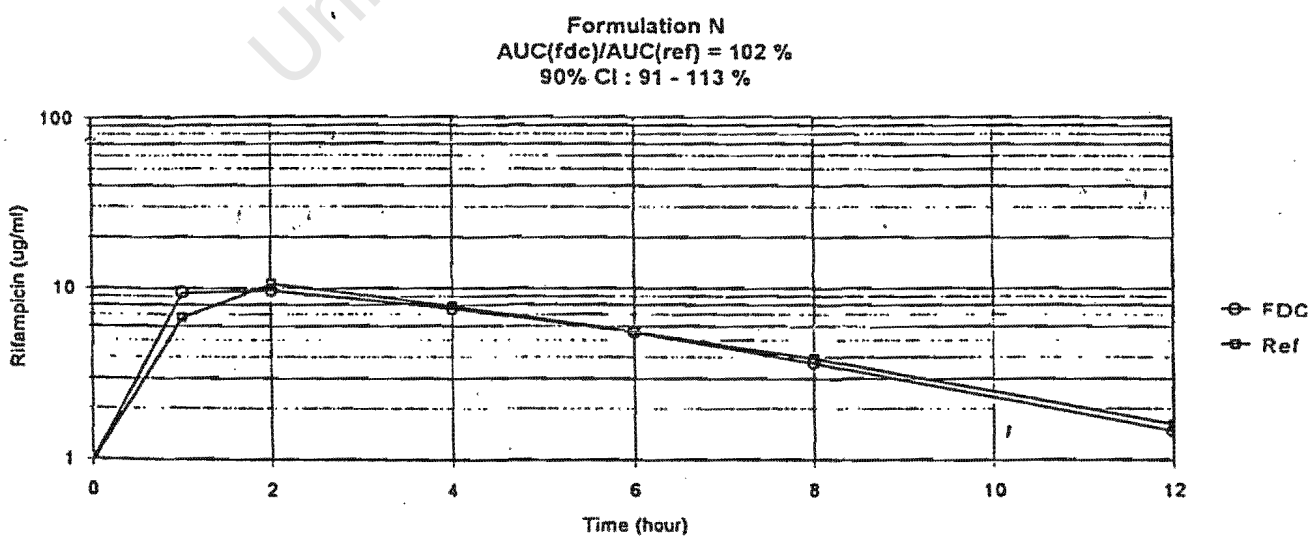

Figure 1 Graph of mean log rifampicin concentration (μg/ml) versus time (hours) in 19 healthy volunteers. The test Formulation N (○), a three-drug fixed-dose combination anti-tuberculosis formulation containing rifampicin (120 mg), isoniazid (50 mg) and pyrazinamide (300 mg) and the reference formulations (□) were administered in equivalent doses in a randomised cross-over study with a 1 week washout period.

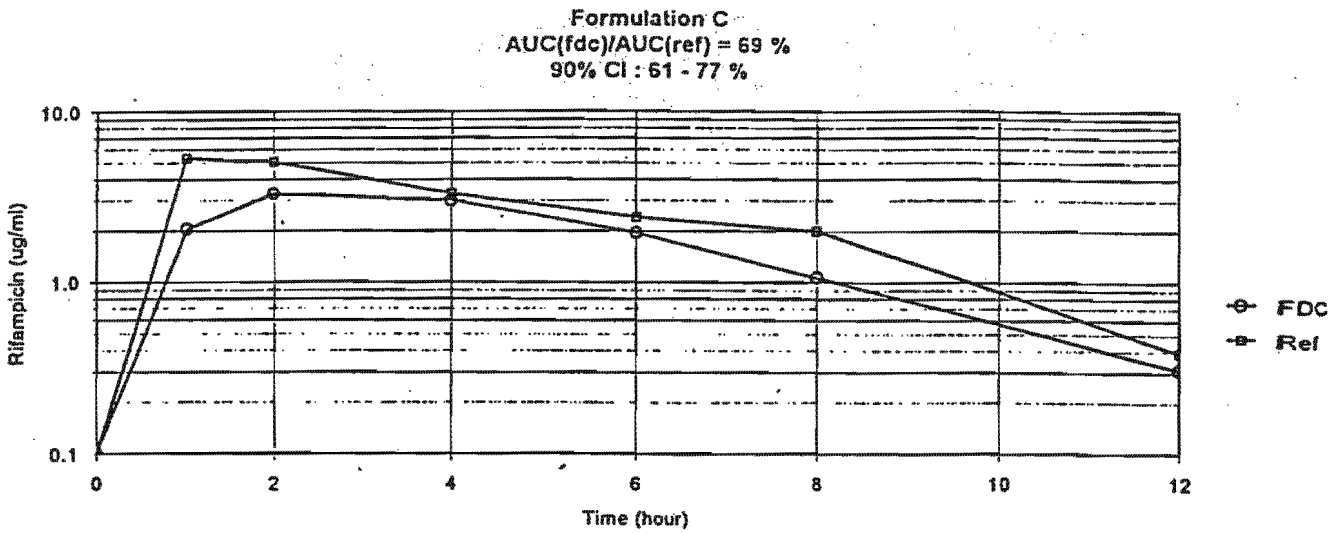

Figure 2 Graph of mean log rifampicin concentration (μg/ml) versus time (hour) in 22 healthy volunteers. The test Formulation C (○), a three-drug fixed-dose combination anti-tuberculosis formulation containing rifampicin (225 mg), isoniazid (150 mg) and pyrazinamide (500 mg) and the reference formulations (□) were administered in equivalent doses in a randomised cross-over study with a 1 week washout period.

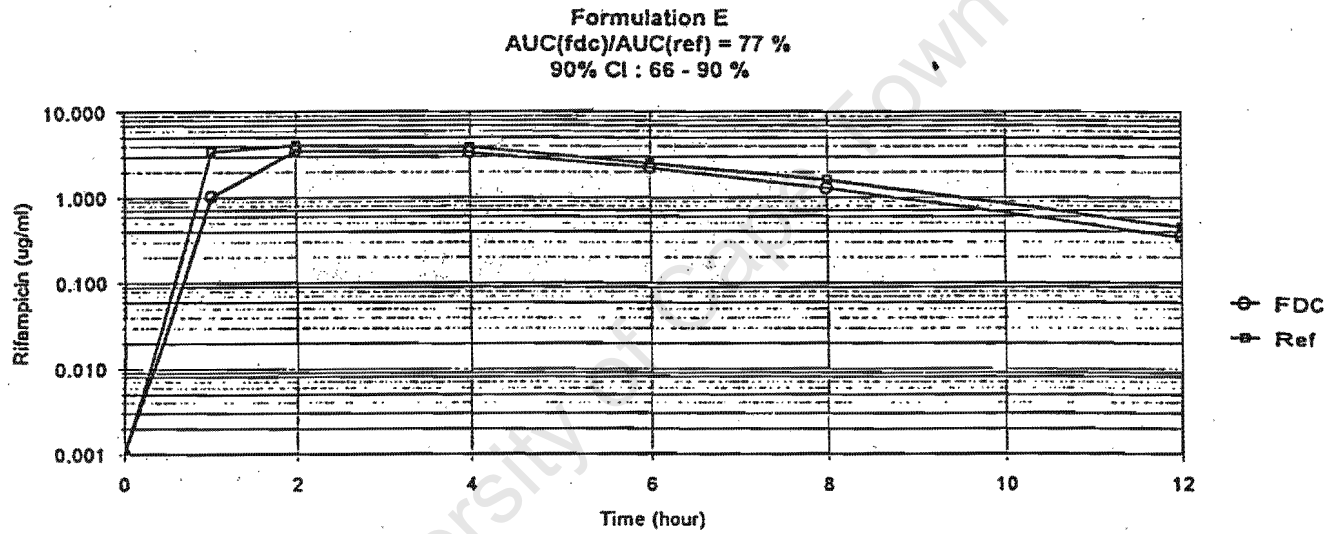

Figure 3 Graph of mean log rifampicin concentration (μg/ml) versus time (hour) in 20 healthy volunteers. The test Formulation E (○), a four-drug fixed-dose combination anti-tuberculosis formulation containing rifampicin (225 mg), isoniazid (150 mg), pyrazinamide (750 mg) and ethambutol (400 mg) and the reference formulations (□) were administered in equivalent doses in a randomised cross-over study with a 1 week washout period.

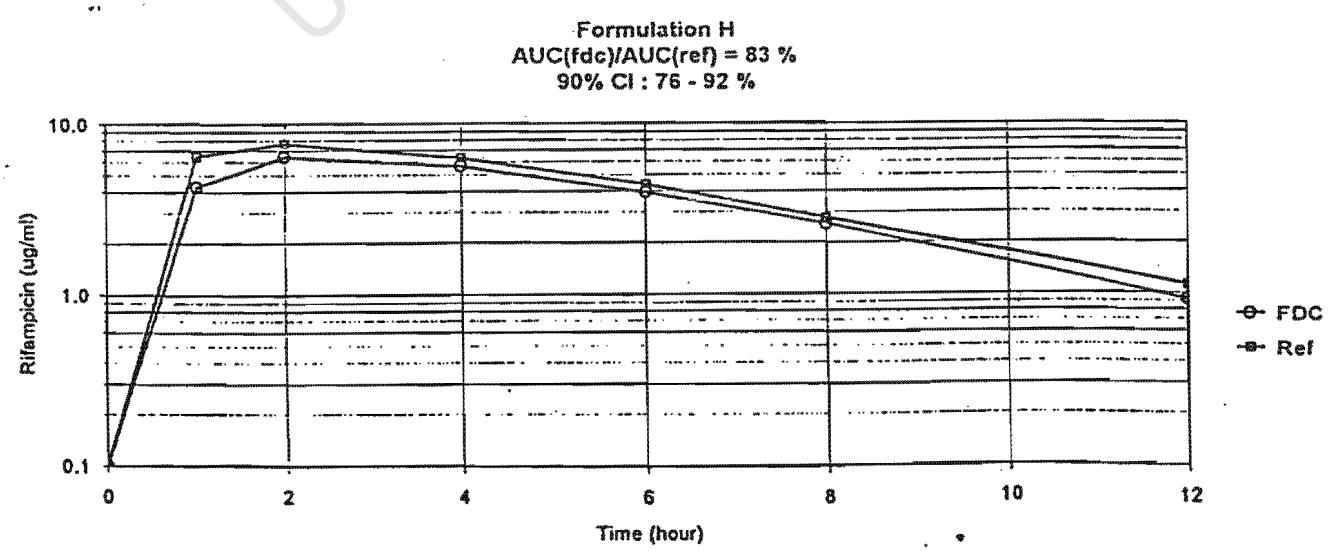

Figure 4 Graph of mean log rifampicin concentration (μg/ml) versus time (hour) in 21 healthy volunteers. The test Formulation H (○), a four-drug fixed-dose combination anti-tuberculosis formulation containing rifampicin (150 mg), isoniazid (100 mg), pyrazinamide (500 mg) and ethambutol (267 mg) and the reference formulations (□) were administered in equivalent doses in a randomised cross-over study with a 1 week washout period.

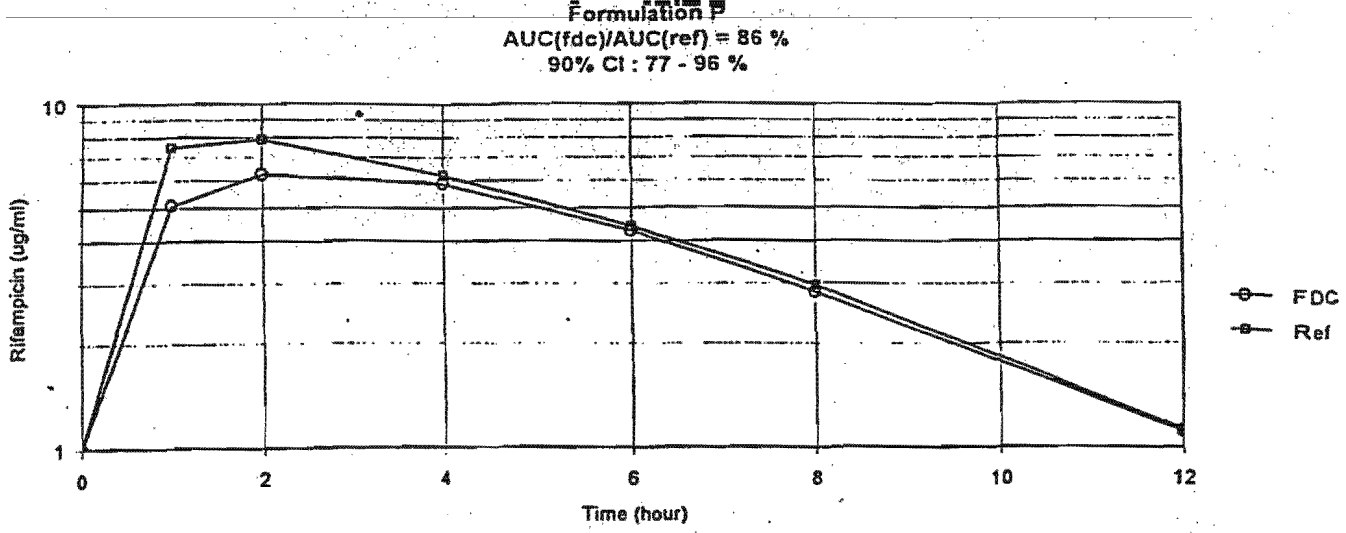

Figure 5 Graph of mean log rifampicin concentration (μg/ml) versus time (hour) in 22 healthy volunteers. The test Formulation P (○), a two-drug fixed-dose combination anti-tuberculosis formulation containing rifampicin (300 mg) and isoniazid (150 mg) and the reference formulations (□) were administered in equivalent doses in a randomised cross-over study with a 1 week washout period.

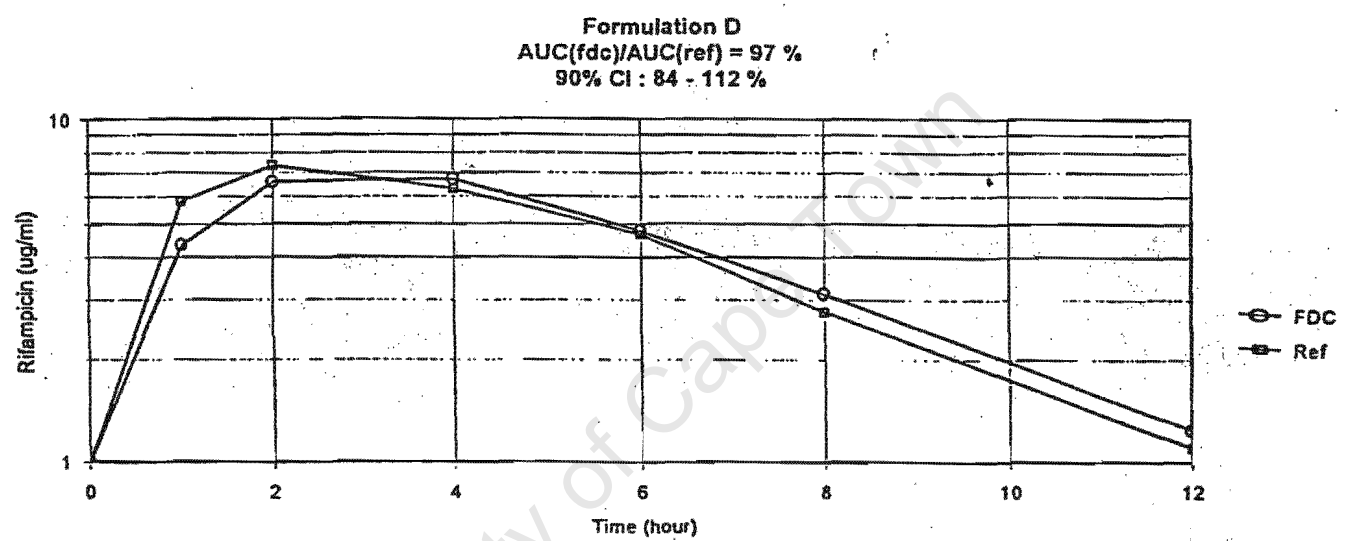

Figure 6 Graph of mean log rifampicin concentration (μg/ml) versus time (hour) in 20 healthy volunteers. The test Formulation D (○), a two-drug fixed-dose combination anti-tuberculosis formulation containing rifampicin (150 mg) and isoniazid (100 mg) and the reference formulations (□) were administered in equivalent doses in a randomised cross-over study with a 1 week washout period.

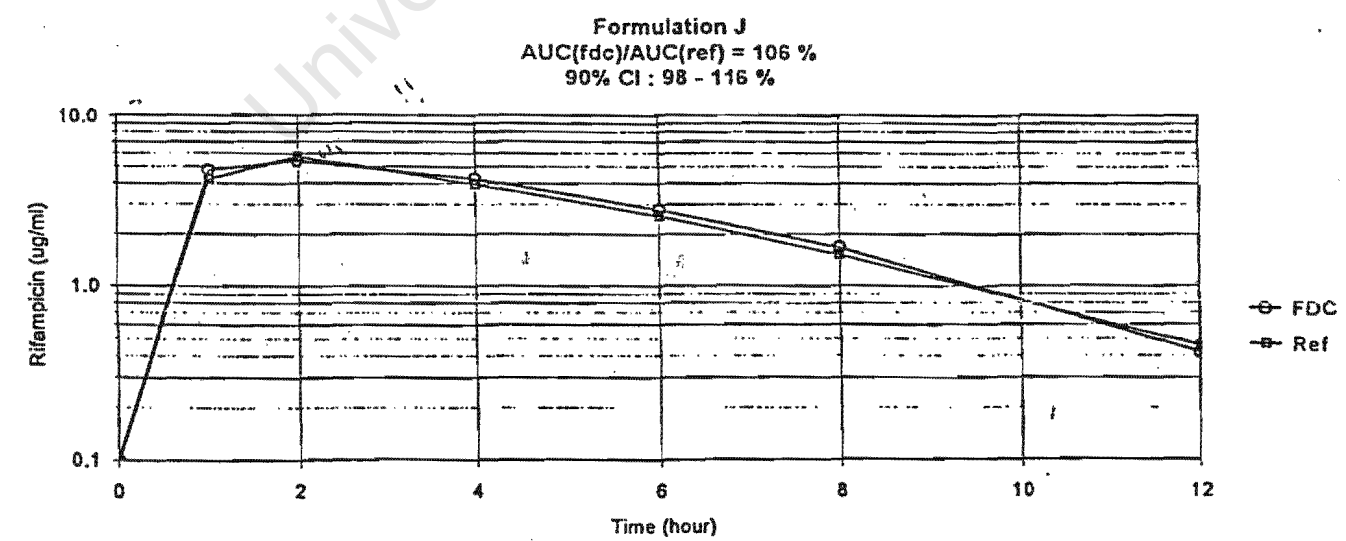

Figure 7 Graph of mean log rifampicin concentration (μg/ml) versus time (hour) in 22 healthy volunteers. The test Formulation J (○), a three-drug fixed-dose combination anti-tuberculosis formulation containing rifampicin (225 mg), isoniazid (150 mg) and pyrazinamide (750 mg) and the reference formulations (□) were administered in equivalent doses in a randomised cross-over study with a 1 week washout period.

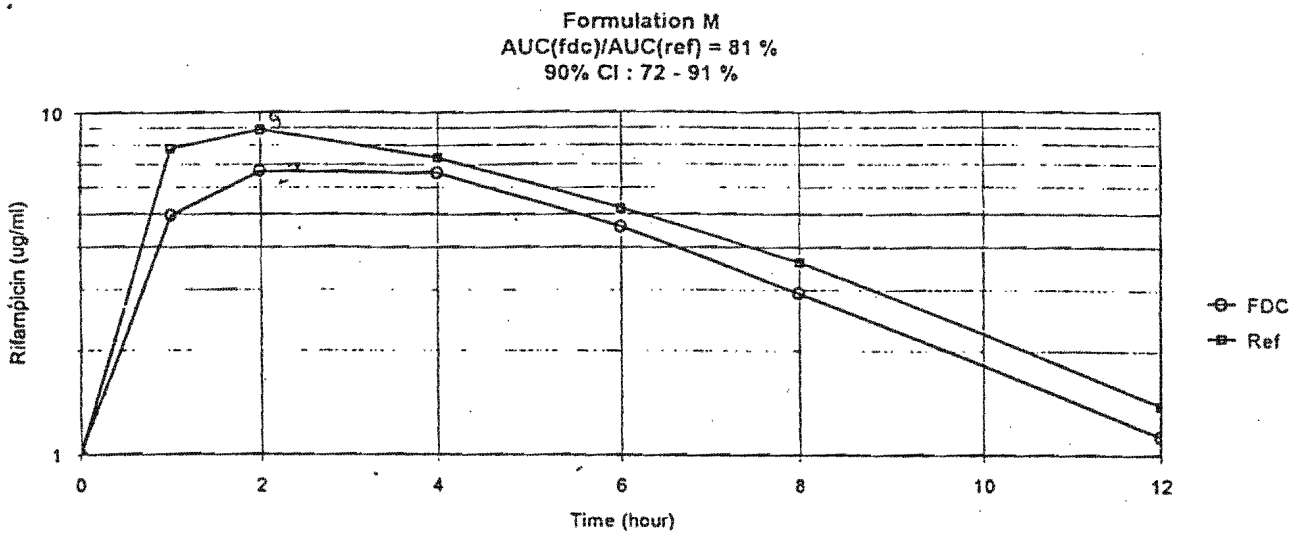

Figure 8 Graph of mean log rifampicin concentration (μg/ml) versus time (hour) in 21 healthy volunteers. The test Formulation M (○), a three-drug fixed-dose combination anti-tuberculosis formulation containing rifampicin (120 mg), isoniazid (80 mg) and pyrazinamide (250 mg) and the reference formulations (□) were administered in equivalent doses in a randomised cross-over study with a 1 week washout period.

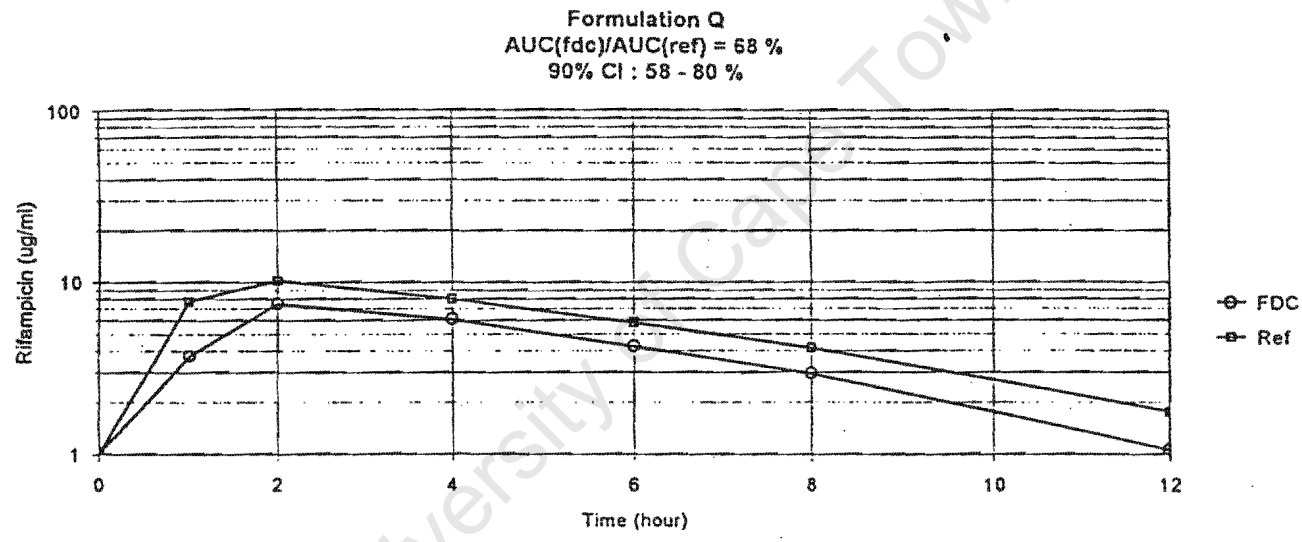

Figure 9 Graph of mean log rifampicin concentration (μg/ml) versus time (hour) in 21 healthy volunteers. The test Formulation Q (○), a two-drug fixed-dose combination anti-tuberculosis formulation containing rifampicin (150 mg) and isoniazid (100 mg) and the reference formulations (□) were administered in equivalent doses in a randomised cross-over study with a 1 week washout period.

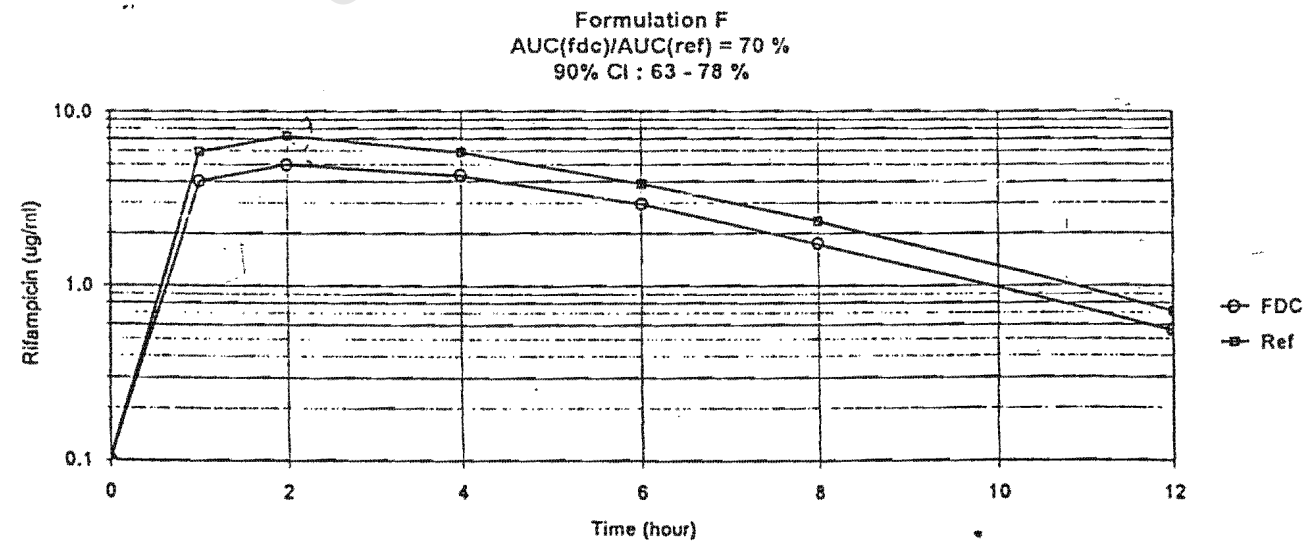

Figure 10 Graph of mean log rifampicin concentration (μg/ml) versus time (hour) in 22 healthy volunteers. The test Formulation F (○), a three-drug fixed-dose combination anti-tuberculosis formulation containing rifampicin (225 mg), isoniazid (150 mg) and pyraznamide (750 mg) and the reference formulations (□) were administered in equivalent doses in a randomised cross-over study with a 1 week washout period.

The therapeutic margin of rifampicin activity is low.[16] Hence any reduction in bioavailability has serious implications for both the individual patient as well as for national tuberculosis control programmes due to potential treatment failure and the selection of drug-resistant mutants.

The primary criterion used for assessment of bioequivalence was the AUC, as recommended by international regulatory bodies such as the United States Food and Drug Administration. In this study, a restricted screening protocol utilising six blood collection time points was used,[11] with the collection of an additional blood sample at 12 hours. In view of the restricted sampling at the time of the estimated C_{max}, this parameter was considered to be of lesser importance in the assessment of bioequivalence compared to AUC. This is apparent for formulation P, which achieved a C_{max} within the acceptable bioequivalence range but AUCs that were not acceptable.

Table 2 shows that extending the duration of blood collection from 8 to 12 hours provided only a small gain in precision. This is in accordance with previous evidence presented by McIlleron et al.[11] Furthermore, the concurrence of the results from the parametric and non-parametric methods in this study is reassuring.

It is clearly important to extend these studies to include other FDCs at present being widely used with unknown bioavailability. Bioequivalence studies have not been a requirement for registration of anti-TB

Table 2 Rifampicin bioequivalence (%) from fixed-dose combination (FDC) anti-tuberculosis drug formulations relative to the drugs administered as separate formulations—non-parametric and parametric analyses on AUC_{0-8}, AUC_{0-12} and C_{max}

		Non-parametric analysis Hauschke analysis			Parametric analysis Schuirmann approach		
Study code		AUC_{0-8}	AUC_{0-12}	C_{max}	AUC_{0-8}	AUC_{0-12}	C_{max}
Formulation N	Median/mean	101	100	104	104	102	105
n = 19	90% CI	93–115	92–109	91–120	91–117	91–113	91–121
	$P < 80$				0.001	0.001	0.002
	$P > 125$				0.028	0.008	0.06
Formulation C	Median/mean	70	69	68	68	69	66
n = 22	90% CI	62–77	62–77	56–78	61–76	61–77	57–77
	$P < 80$				0.990	0.986	0.981
	$P > 125$				<0.001	<0.001	<0.001
Formulation E	Median/mean	75	76	81	75	77	81
n = 20	90% CI	62–90	65–92	70–99	64–88	66–90	69–96
	$P < 80$				0.75	0.67	0.44
	$P > 125$				<0.001	<0.001	<0.001
Formulation H	Median/mean	82	84	80	82	83	82
n = 21	90% CI	75–90	76–92	71–93	74–91	76–92	73–91
	$P < 80$				0.35	0.23	0.39
	$P > 125$				<0.001	<0.001	<0.001
Formulation P	Median/mean	87	88	91	83	86	87
n = 22	90% CI	77–96	79–98	82–98	73–94	77–96	76–99
	$P < 80$				0.3	0.15	0.16
	$P > 125$				<0.001	<0.001	<0.001
Formulation D	Median/mean	95	97	97	94	97	98
n = 20	90% CI	80–107	83–112	82–116	81–110	84–112	82–117
	$P < 80$				0.03	0.015	0.03
	$P > 125$				0.006	0.01	0.03
Formulation J	Median/mean	106	106	100	107	106	101
n = 22	90% CI	98–116	97–116	90–112	98–116	98–116	90–114
	$P < 80$				<0.001	<0.001	<0.001
	$P > 125$				0.01	0.01	0.01
Formulation M	Median/mean	80	78	73	82	81	76
n = 21	90% CI	71–93	72–91	64–88	72–94	72–91	66–86
	$P < 80$				0.35	0.41	0.77
	$P > 125$				<0.001	<0.001	<0.001
Formulation Q	Median/mean	65	66	70	68	68	72
n = 21	90% CI	57–80	57–79	58–84	57–81	58–80	59–87
	$P < 80$				0.94	0.95	0.82
	$P > 125$				<0.001	<0.001	<0.001
Formulation F	Median/mean	73	73	72	70	70	69
n = 22	90% CI	65–79	64–78	66–79	63–78	63–78	61–78
	$P < 80$				0.98	0.97	0.98
	$P > 125$				<0.001	<0.001	<0.001

AUC = area under the curve; C_{max} = maximal serum concentration; 90%CI = 90% confidence interval.

formulations despite WHO/IUATLD statements to this effect. Instead, formulations have been registered on the strength of dissolution and stability data alone. The implications for TB control programmes are extremely serious and warrant urgent attention.

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