Formulation and bioequivalence testing of fixed-dose combination orally disintegrating tablets for the treatment of tuberculosis in paediatric population

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Abstract

Tuberculosis (TB) is believed to affect around 10 million people worldwide. Treatment for TB includes isoniazid and rifampicin, with fixed-dose combination (FDC) recommended for improved patient compliance. Similarly, orally disintegrating tablets (ODTs) are an increasingly popular dosage form that aid compliance since they do not require swallowing. In this study ODTs of isoniazid and rifampicin, either as discrete or FDC doses, were formulated and bioequivalence between single and combination doses compared using *in vitro* and *in silico* approaches. Dissolution profiles were compared using FDA advised difference (f_1) and similarity (f_2) testing in biorelevant media. Rifampicin release from FDCs decreased by approximately 15% in fed-state media (failed f_1 and f_2), which was attributed to enhanced rifampicin degradation in the presence of isoniazid at lower pH. Apparent permeability (P_{app}) values derived from Caco-2 transport studies were included alongside dissolution results into a physiologically based pharmacokinetic (PBPK) model, to simulate *in vivo* bioavailability in healthy subjects. Models showed no difference in bioavailability between formulations or dosing (fasted or fed) state, despite the failures in dissolution-based bioequivalence testing, highlighting shortcomings in f_1 and f_2 assessment and the strength of PBPK models.

Introduction

Recognised as one of humankind's oldest diseases, with evidence of cases dating back more than 5000 years [1], tuberculosis (TB) remains a major cause of morbidity and mortality. Today there are an estimated 9.6 million TB cases worldwide, with the disease claiming 1.5 million deaths in 2014 alone [2]. Since 2000 the incidence of TB has fallen by 18%, at an average rate of 1.5% per year, with effective treatment within this time frame saving an estimated 43 million lives [2].

TB is an infectious disease caused by the aerobic bacterium *Mycobacterium tuberculosis* (MTB). Transmission occurs through aerosolisation of the bacterium into droplet nuclei by coughing, sneezing or talking [3]. Inhalation of the organism into the alveoli leads to respiratory infection, that if spreads, causes extrapulmonary tuberculosis, which can involve any organ system in the body [4]. Pulmonary tuberculosis, the most common presentation, is avoided in most cases of exposure through mucociliary clearance [5], or failing that through the successful activity of phagocytic alveolar macrophages, resulting in symptomless latent tuberculosis [6]. Around 5% of TB infections progress to the active form of the disease within two years, with about 10% of latent cases reactivating at some point later in life [3]. TB outcome is dependent on a multitude of factors, most prominent of which is the immunocompetence of the individual, which itself depends on various intrinsic and extrinsic factors such as the hosts genetics and nutritional state, respectively [7,8].

Clinical manifestation of TB depends on the site of infection. Pulmonary TB, historically referred to as consumption or pthisis, classically manifests as severe wasting [8], as well as cough, haemoptysis, chest pain, dyspnoea, malaise, fatigue, low-level fever and night sweats [9]. Extrapulmonary TB can include the same symptoms as pulmonary TB, with a wide range of additional symptoms based upon the site of infection, such as meningitis (CNS), lymphadenitis (lymphatic), arthritis (skeletal) and haematuria (renal) [10].

Various social, environmental and biological risk factors determine the risk of TB contraction. Risks for infection and progression to disease are distinctly different; infection risk involves extrinsic factors including social and behavioural risks (alcohol, smoking and pollution), source infectiousness and proximity (including overcrowding and length of exposure), whereas risk of progression to disease is endogenous to the host [11]. Immunosuppressive conditions accelerate progression to active disease, with HIV being especially potent [12]. Impaired immune response as a result of malnutrition is also known to increase the risk of TB [13], whilst a strong socioeconomic association with the disease exists, with the poorest experiencing the greatest risk [14]. Children also present an increased susceptibility to TB development, which is greater still before the age of 2 and after age 10 [15]. Other risk factors for progression to disease include diabetes, alcohol, smoking and indoor air pollution [11].

Isoniazid and rifampicin form the basis of front-line treatment for TB [2], with both drugs included in the WHO Model List of Essential Medicines and Essential Medicines for Children. Isoniazid (BCS class I/III [16]) is a pro-drug that requires activation by catalase-peroxidase enzyme (KatG), which is endogenous to MTB [17]. The drug inhibits the synthesis of mycolic acids, essential components of the bacterial cell wall and at therapeutic doses is bactericidal against actively growing intra and extra cellular MTB [18]. Rifampicin (BCS class II [19]) also displays a bactericidal effect on MTB, by inhibition of transcription through high-affinity binding to the β-subunit of bacterial DNA-dependent RNA polymerase [20]. Rifampicin is highly effective against TBM through its ability to readily diffuse into tissues, cells and bacteria [21]. The tendency of rifampicin to degrade substantially when combined with isoniazid in acidic media is a well-recognised complication when considering combination of the two drugs in solid oral-dosage forms [22].

The first-line recommended oral drug regimen for treatment of drug susceptible TB involves isoniazid, rifampicin, pyrazinamide and ethambutol for 2 months, followed by isoniazid and rifampicin for 4 months, with the regimen altering due to drug or multi-drug resistance [23]. Treatment for extrapulmonary TB does not differ, except in some cases where duration of therapy is extended [24]. Recommended doses for treatment of children differ compared to adults. Fixed-dose combinations (FDCs) are recommended for TB treatment of both adults and children [23], however FDCs currently on the market do not correspond to appropriate doses for children [24]. FDCs for TB treatment have not been shown to alter efficacy, drug resistance or adverse effects or events when compared to single-dose [24]. Furthermore, whilst FDCs have not provided evidence for

improvement of treatment outcomes, their use simplifies TB therapy, with some evidence for an increase in patient satisfaction [25].

Orally disintegrating tablets (ODTs) are an increasingly popular dosage form that improve compliance for patients with dysphagia, a difficulty swallowing, particularly prevalent in paediatric and geriatric populations, institutionalised and psychiatric patients and sufferers of nausea and vomiting [26]. ODTs are designed to rapidly disintegrate in contact with saliva within the oral cavity, removing the need for swallowing and coadministration with water. Market studies have shown ODTs to be popular amongst patients, with over 50% preferring them to other dosage forms (such as regular tablets and liquids) and 70% of consumers declaring they would request ODTs from their physician [27].

In order for a new generic formulation to be approved it needs to demonstrate bioequivalence with a reference branded product. A bioequivalent drug will display comparable bioavailability and thus *in vivo* performance (efficacy and safety) [28]. Bioequivalence can be assumed in the absence of clinical trials, if there is no significant difference in the rate and extent to which the active pharmaceutical ingredient (API) becomes available within the systemic circulation, when compared with the reference product [29]. Bioequivalence testing may also be applied in the assessment of FDCs [28]. For immediate release formulations bioequivalence can be determined by comparison of *in vitro* dissolution profiles using FDA recommended difference factor (f_1) and similarity factor (f_2) testing, for biowaiver applications [29]. Comparison testing is not deemed necessary if test products display greater than 85% dissolution within 15 mins, given that the API falls within BCS class I or III (although class III carries stricter requirements) [28]. The extension of biowaivers to BCS class II compounds is a topic of much discussion [30].

Pharmacokinetic modelling and simulation has become an established tool over the past 20 years to predict drug pharmacokinetics in humans and assess the effect of intrinsic and extrinsic factors on drug exposure. Physiologically based pharmacokinetic (PBPK) models define tissues and organs as compartments, with parameters based upon decades of knowledge of body fluid dynamics. PBPK models consider ADME processes throughout all compartments to estimate the pharmacokinetic profile of a drug at a target tissue or organ [31]. As such, PBPK models have become a powerful tool for the prediction of oral drug absorption through integration of common *in vitro* drug-specific information, alongside a variety of physiological descriptions of the population groups (e.g. age, weight, height, tissue perfusion, drug metabolising enzyme abundance and ontogeny) [32]. PBPK modelling is often exploited for prediction of oral drug absorption and to study formulation changes [33] or FDCs [34]. There is also an effort to apply PBPK modelling to predict the bioequivalence of new generic formulations with reference drugs [35].

An FDC ODT for isoniazid and rifampicin could potentially improve patient compliance and be particularly beneficial in developing areas with little to no access to water. The use of a paediatric relevant dose would be valuable given the current lack of support and the widely reported and supported applicability of ODTs to enhance compliance in paediatric populations [36]. Similarly, improved clinical outcomes from FDCs, due primarily to improved adherence as a result of reduced pill burden, are well documented [37].

This work demonstrates the ability of PBPK modelling and clinical trial simulations to overcome the challenge of drug testing in paediatric populations. Specifically, this study focuses on the development of isoniazid and rifampicin single and FDC ODT formulations at paediatrically relevant doses. *In vitro* drug dissolution and permeability data was used to predict drug pharmacokinetics for *in silico* models, in order to investigate API bioequivalence between single and fixed-dose formulations.

Materials and Methods

Materials

Isoniazid and rifampicin were purchased from Molekula Ltd (UK). Pearlitol® Flash (mannitol-starch copolymer) was obtained from Roquette Pharma (France), whilst Avicel PH-102 micro-crystalline cellulose (MCC) and sodium stearyl fumarate (SSF) were purchased from FMC BioPolymer (USA).

Biorelevant FaSSIF/FeSSIF/FaSSGF Instant Powder was purchased from biorelevant.com (UK). Sodium hydroxide, sodium chloride, sodium phosphate and glacial acetic acid for biorelevant media were obtained from Sigma-Aldrich (UK). Acetonitrile (ACN) and methanol (HPLC-grade) were obtained from Fisher Scientific (UK).

For cell culture media DMEM was purchased from Lonza (UK), fetal bovine serum (FBS), gentamicin (10 mg/ml), Fungizone (amphotericin B 250 µg/ml), HBSS and penicillin/streptomycin (10,000 U/ml) were all purchased from Gibco (Thermo Fischer Scientific, UK). Trypsin-EDTA solution (0.25%) was procured from Sigma-Aldrich (UK).

Tablet production

Direct compression of tablets (500 mg) at a compaction force of 2.2 tons was performed on an Atlas T8 automatic press (SPECAC, UK), using a 13mm round, flat-faced die. Tablets were produced under ambient conditions.

Disintegration testing

Disintegration testing was performed in accordance with US pharmacopeia monograph ([701] disintegration). An Erweka ZT3, Appartebau, GMBH (Germany) was used as disintegration apparatus and 800 ml distilled water maintained at 37˚C was used as the disintegration media. Tablets were measured individually by placing in the basket rack and the time taken for the tablets to disintegrate without leaving any solid residue in the rack, recorded. Disintegration time was measured in triplicate.

Friability

Tablet friability was determined on 6 tablets using an F2 friability tester (Sotax, Switzerland). Tablets were placed inside the drum and rotated at 25 rpm for a total of 100 revolutions. Dust was removed pre and post testing to remove excess powder that would contribute to tablet mass. Friability was calculated and expressed as % tablet weight loss from initial tablet weight.

Tablet hardness

A Tablet Hardness Tester TBF1000 (Copley Scientific, UK) was used to measure the radial crushing strength (hardness) of tablets in triplicate.

High performance liquid chromatography (HPLC)

HPLC was performed on an Agilent 1260 series (Agilent Technologies, USA), comprising a quarternary pump, Infinity VWD and autosampler. Analysis was conducted on a reversed-phase Gemini C18, 150 x 4.6 mm, 110Å, 5µm column (Phenomenex, UK). Protocols were developed, calibrated and validated for both isoniazid and rifampicin alone and in combination.

Separations were achieved using either (Type 1) H_2O, 0.1% (v/v) TEA, 0.1% (v/v) TFA or ACN at different ratios as the mobile phase. Ascorbic acid (0.5 mg/ml) was included as an antioxidant to prevent rifampicin degradation [38]. Isoniazid separation was performed with an isocratic mobile phase of H_2O: ACN (90:10 v/v), a flow rate of 1 ml/min and a wavelength of 254 nm. Rifampicin

separation was achieved using an isocratic mobile phase of TFA: ACN (45:55 v/v), a flow rate of 1 ml/min and a wavelength of 254 nm. Separation of isoniazid and rifampicin in combination required a mobile phase of TEA: ACN delivered at a gradient (95:5 to 20:80 v/v), with a flow rate of 1 ml/min and a wavelength of 254 nm. An injection volume of 20 µl was used throughout.

HPLC method validation involved assessment of precision through intra-day variation, accuracy by multilevel recovery studies, instrument precision, linearity and limit of detection and quantification (LOD and LOQ). Stock solutions (1 mg/ml) of each drug were prepared in mobile phase from which dilutions and subsequently two-fold serial dilutions were prepared to form a calibration curve.

Dissolution testing

API dissolution from ODTs in 900 ml biorelevant media (FaSSIF/FeSSIF/FaSSGF instant powder, biorelevant.com, UK) was tested in both fasted state simulated intestinal fluid (FaSSIF) and fed state simulated intestinal fluid (FeSSIF), at pH 6.5 and 5 respectively and maintained at 37°C. An ERWEKA DT 600 USP 2 paddle apparatus (Germany) was used at a paddle speed of 50 rpm [39]. 5ml samples were taken over 2 h, replacing with 5 ml fresh media to simulate sink conditions. API dissolution was measured using HPLC and corrected for % dose dissolved.

Cell culture

Prior to seeding, cells were trypsinised (2.5 ml) from 75-cm^2 cell culture flasks (Corning, USA) on which they had been grown (80% confluence), after washing with HBSS. Caco-2 cells (passage 54-58) were seeded onto Transwell (Corning, USA) semi-permeable membrane supports (12-well, 1.12 cm^2, 0.4 µm pore size) at a density of $8x10^4$ cells/cm^2. Cells were maintained in Dulbecco's modified Eagle's minimal essential medium (DMEM) containing L-glutamine (4 mM) and glucose (4.5 mg/ml), and supplemented with (v/v) 10% fetal bovine serum, 1% penicillin/streptomycin, 1% non-essential amino acids, amphotericin B (0.5 µg/ml) and gentamicin (20 µg/ml). Media was changed every 2-3 days and transwells cultured at 37°C, 5% CO_2 for 21 days, after which transport studies were performed.

Caco-2 transport studies

Caco-2 cells were purchased from the European Collection of Authenticated Cell Cultures (ECACC) via Public Health England. Caco-2 monolayers were used for transport studies between 21 and 24 days post-seeding. Drug absorption through Caco-2 monolayers was measured for isoniazid and rifampicin alone and in combination in both the apical to basolateral (A-B) and basolateral to apical (B-A) directions (n=3). Transport studies were carried out in DMEM (37°C) containing 10 mM HEPES (pH 7.4), with 0.5 ml and 1.5 ml in the A and B compartments, respectively. Samples of 100 µl were removed from the A side and 200 µl from the B side at time points over 2 h, replacing with fresh pre-warmed media (37°C) to mimic sink conditions. For mass balance, samples were taken from the donor compartments at t=0 and t=120 mins.

Isoniazid was administered at a concentration of 20 µg/ml and rifampicin at a concentration of 30 µg/ml. Concentrations used were comfortably within or below previously reported well tolerated concentration ranges for both isoniazid and rifampicin [40]. Cultures were maintained at 37°C and 5% CO_2 throughout the experiment. Samples were analysed by HPLC and apparent permeability (Papp) values were calculated using equation:

$$P_{app} = (dQ/dt)/(C_0 \times A)$$

Where dQ/dt is the mass transfer rate of the compound from the donor to the receiver compartment, C_0 is the initial concentration in the donor chamber and A is the monolayer surface area (cm^2).

Clinical trials simulation

The population-based clinical trials simulator Simcyp (V14) (Certara, USA) was used to simulate the plasma concentration of isoniazid and rifampicin from single API and FDC formulations. Default parameter values for creating a North European Caucasian population were selected [41].

Compound data

Physicochemical information for each API was collated from the literature used to develop compound files (Table 8). Simulations were performed using a minimal-PBPK model. Where uncertainty arose regarding the precise value of compound data parameters, parameter estimation was conducted using the Parameter Estimation Module to optimize parameter values. The ADAM model [42] was assumed for all simulations and the dissolution profile for each formulation (single and FDC) in FaSSIF and FeSSIF was utilised.

Clinical studies

The optimization and validation of the PBPK model was conducted using clinical study results reported in healthy adult subjects. For isoniazid: study 1 included a total dose of 300 mg dosed to 18 healthy volunteers (18-55 years old) [43]; study 2 included a total dose of 300 mg dosed to 22 healthy volunteers [44]; study 3 included a total dose of 300 mg dosed to 20 healthy volunteers (23 ± 1.8 years old) [45]; study 4 included a total dose of 300 mg dosed to 18 healthy volunteers (36.4 ± 10.6 years old) [46]. Studies 1 and 2 were used for model development and studies 3 and 4 utilized for validation.

For rifampicin: study 1 included a total dose of 600 mg dosed to 18 healthy volunteers (18-55 years old) [43]; study 2 included a total dose of 600 mg dosed to 20 healthy volunteers (23 ± 1.8 years old) [45]; study 3 included a total dose of 600 mg dosed to 18 healthy volunteers (36.4 ± 10.6 years old) [46]; study 4 included a total dose of 600 mg dosed to 22 healthy volunteers [44]. Studies 1 and 2 were used for model development and studies 3 and 4 utilized for validation.

Raw data from published human trial plasma concentration profiles was extracted using WebPlotDigitizer 3.10 [47] and, where necessary, parameter estimation was conducted using the validation clinical datasets.

Predictions of API plasma pharmacokinetic profiles were simulated following the oral administration of a single immediate release solid dosage form of 50mg (isoniazid) and 75 mg (rifampicin) dose over a 24 hr period.

To assess the impact of ABCB1 active efflux on rifampicin fraction dose absorbed (fa), we conducted a local sensitivity analysis by varying ABCB1 active transport clearance (CLtrans) (0.1 to 100 µL/min/pmol) and Papp (0.1-100 x10^{-6} cm/s), then assessing the resulting impact on fa.

Statistical analysis

GraphPad PRISM software version 6.01 (USA) was used for data analysis. Ordinary one-way ANOVA was used with Tukey's multiple comparisons test to analyze data for tablet characterization. Unpaired two-tailed t-test was used to determine statistical differences between data sets for pharmacokinetic parameters.

Differences between dissolution profiles of APIs in single dose (reference) and combination (test) were assessed using f_1 and f_2 difference and similarity factor testing, using the equations [48]:

$$f_1 = ([\sum_{t=1}^{n} |R_t - T_t|]/[\sum_{t=1}^{n} R_t]) * 100$$

$$f_2 = 50 * \log([1 + (1/n)\sum_{t=1}^{n} (R_t - T_t)^2]^{-0.5} * 100)$$

Where R_t and T_t are the % drug dissolved value at each time point for the reference and test product respectively and n is the number of time points.

Results and discussion

ODT development

An ODT formulation for rifampicin and isoniazid both alone and in combination was developed, with the requirement that tablets were mechanically robust whilst maintaining rapid disintegration. Round flat faced tablets (500 mg) were produced by direct compression. In order to isolate the effect of combination of APIs, the number of excipients used was kept at a minimum. The formulation consisted of API alongside Na stearyl fumerate (SSF, 0.5% w/w) as a lubricant and Pearlitol as a diluent. Compaction forces were applied at a range of 1-2 T, with hardness values acceptable (>60 N) from a compaction force of 1.2 T and above. Friability values at all compaction forces were high (>1%), with tablets compressed at and below 1.2 T unable to withstand friability testing. Disintegration times at all compaction forces were within 30 s (18-21 s), as recommended by the FDA for ODTs [49], with no significant effect ($p>0.05$) on disintegration with changes in compaction force. At 2 T compaction force tablet hardness peaked at 100.17 ± 7.97 N and friability dropped to 1.97%. Increasing SSF to 1.5% w/w ensured improved lubricant ability whilst maintaining high hardness and a low disintegration time.

To combat high friability MCC was included as a binder [50]. Addition of MCC up to 15% w/w increased hardness ($p>0.01$), compared to 5% and 10%, to 119.50 ± 3.90 N, whilst lowering friability and maintaining rapid disintegration. MCC has excellent binding properties due to its plastic deformation, maximising interparticulate bonding [50] and hydrogen bond formation between adjacent molecules [51], whilst mechanical interlocking has also been proposed as a mechanism [52]. The high intraparticle porosity of MCC promotes rapid penetration of water through capillary action and is responsible for its ability to enhance disintegration [50]. Raising compaction force to 2.2 T lowered tablet friability to 1.10-0.85 %, maintained rapid disintegration and raised hardness to as high as 151.17 ± 4.48 N. Formulation composition is shown in Table 1 and characterisation of formulations is shown in Table 2.

HPLC protocol validation

Linearity test solutions were prepared from stocks at six concentrations ranging from 100 to 1.5625 µg/ml. Validation of protocols by intraday studies for isoniazid, rifampicin and isoniazid/rifampicin combination are shown in Table 3. Instrument precision, tested for by six consecutive injections of the same sample (100 µg/ml), ranged from 0.08% to 0.87%. All protocols showed good method accuracy and precision. Method accuracy is demonstrated by multilevel recovery, ranging from 100 µg/ml to 6.25 µg/ml. Accurate recovery was exhibited at each concentration for both APIs, ranging from 98.03% to 101.98%, with mean recovery values shown. Relative standard deviation (RSD) values representing intraday precision for isoniazid, rifampicin and isoniazid/rifampicin were low, ranging from 0.51 to 2.40 %, with mean values displayed. LOQ and LOD values for isoniazid and rifampicin alone were at or below 0.80 and 0.24 µg/ml, respectively. LOQ and LOD values for isoniazid in combination were lower still, whilst rifampicin in combination showed the greatest LOQ and LOD of 1.18 and 0.36 µg/ml, respectively.

Dissolution

Drug release from ODTs was tested in FaSSIF and FeSSIF media (Table 4). Rapid and complete isoniazid dissolution from single dose (99.24%) and FDC (100.65%) in FaSSIF (Figure 1) was observed. Difference testing showed dissolution profiles were equivalent (f_1=14.17) however similarity testing indicated differences between both profiles (f_2=32.79). Despite this, isoniazid dissolution exceeded 85% within 15 mins. In FeSSIF (Figure 2) similar drug release profiles for isoniazid are seen from both single and FDC formulations, with dissolution peaking at 100.12% and 101.52%, respectively and both formulations exceeding 85% dissolution by 5 mins. Values for f_1 and f_2 testing show no difference between the two dissolution profiles.

Rifampicin dissolution from single and FDC formulations in FaSSIF (Figure 3) was comparable based on f_1 and f_2 testing, with complete dissolution of 100.63% from single dose, whilst dissolution from FDC peaked at 91.91%. Dissolution profiles for rifampicin from single and FDC in FeSSIF (Figure 4) were deemed different, failing f_1 and f_2 testing. Rifampicin was rapidly

released from single dose, showing >85% dissolution by 5 mins, peaking at 98.26%, however in combination rifampicin release was retarded, with a maximum dissolution after 1 h of 85.32%. This observed drop in dissolution is likely a result of degradation, given the complete release seen from the single dose formulation and the well documented enhanced degradation of rifampicin in the presence of isoniazid under acidic conditions, in this instance pH 5.

Permeability studies

Transepithelial electrical resistance (TEER) values for Caco-2 cells over 21 days plateau from day 18, showing a resistance of 1351.1 ± 88.6 $\Omega \cdot cm^2$ by day 21 post-seeding. Isoniazid and rifampicin transport across Caco-2 monolayers alone and in combination was measured in A-B and B-A directions. P_{app} values are summarised for each drug and drug combination in Table 5.

Isoniazid was readily absorbed across Caco-2 monolayers from both A-B and B-A directions, exhibiting an efflux ratio of 1.18 indicating passive diffusion. Similar permeability was displayed for isoniazid in combination with rifampicin, with an efflux ratio of 1.19. Rifampicin P_{app} values suggested active efflux of the compound, with efflux ratio values of 4.33 and 2.61 from single and combination respectively. Active efflux of rifampicin across Caco-2 monolayers has previously been indicated [53].

Clinical trials simulation

The initial simulation of the kinetics of isoniazid (derived from data presented in Table 8) were used to optimize the human jejenum effective permeability and volume of distribution at steady state (P_{eff} and V_{ss}, respectively) from clinical data sets 1 and 2 for each API. P_{eff} describes a prediction of human absorption rate constants (ka), whereas V_{ss} values describe a conversion factor (mass to concentration) and the tissue distribution of the API. Optimized P_{eff} and V_{ss} were estimated as 10.23 $x10^{-4}$ cm/s and 0.63 L/kg.

Use of passive permeability data (Table 5) to mechanistically model the absorption of rifampicin did not capture the absorption kinetics of rifampicin. Very little data exists which supports the notion that rifampicin undergoes active transport, while the reported Fa of >0.9 [54] would support the notion that no active efflux transporter pathways exist which impact upon the oral bioavailability of rifampicin. However, to assess the impact of potential active efflux on rifampicin absorption, a sensitivity analysis was conducted where active intestinal efflux was attributed to ABCB1 (P-glycoprotein) and the impact of variation in passive permeability (P_{app}) and active efflux transporter clearance (CL_{trans}) on rifampicin Fa was assessed (Figure 5). Assuming rifampicin is a highly permeable compound (human jejunum effective permeability, P_{eff}, > $1x10^{-4}$ cm/s; Simcyp predicted P_{eff}: 2.15 $x10^{-4}$ cm/s) (BCS Class II), active efflux would only impact upon rifampicin Fa under conditions of high efflux (>10 µL/min/pmol). However, for our measured P_{app} (0.137 $x10^{-6}$ cm/s) the resultant P_{eff} is 0.19 $x10^{-4}$ cm/s and would classify rifampicin as a low permeability compound. Furthermore, transporter clearance in excess of 1 µL/min/pmol would impact upon the overall Fa of rifampicin. With this in mind, it was decided to utilise the default optimised rifampicin compound file (see Table 8) within Simcyp without alteration of the absorption kinetics.

Subsequent validation of isoniazid and rifampicin using validation data sets 3 and 4 for each API was successful and generally centred around the mean simulated profiles and were within the 5th and 95th percentiles of the simulated profiles (see Figures 6 and 7).

Simulations to predict the *in vivo* performance of ODTs in healthy volunteers were used to compare the bioavailability between single and FDC formulations under fasted and fed conditions, using the dissolution data. For isoniazid, the formulation state (single or combined) or dosing state (fasted or fed) had no statistically significant impact on pharmacokinetics (Figure 8 a and b). Isoniazid plasma concentrations reached a geometric mean C_{max} of 0.70-0.74 ng/ml in all conditions (Table 6), yielding a median AUC in the range of 4.05-4.24 ng/ml.h.

At the level of the small-intestine Fa for isoniazid correlated with dissolution profiles, showing no significant differences between single and combination formulations, with values of 0.98 ± 0.02 and 0.97 ± 0.03 (fasted) and 0.99 ± 0.04 and 0.96 ± 0.05 (fed), respectively.

Fa values for rifampicin were equivalent between formulation states at 0.94 in fasted subjects; likewise, no difference was seen in Fa for fed subjects, with values of 0.94 for both single and combination doses. These results imply that permeation across the intestinal epithelial membrane was not rate limiting, casting doubt on the ability of f_1 and f_2 factor testing in this instance to predict bioequivalence. Rifampicin plasma profiles similarly showed no statistically significant difference ($p>0.05$) in pharmacokinetic parameters between single and combination doses in fasted subjects (Figure 8c). Rifampicin plasma concentrations (Table 7) in FDCs (irrespective of dosing state) demonstrated higher AUCs (9.26 ng/ml.h) compared to single formulations (8.80 ng/ml.h). Furthermore, geometric mean C_{max} was generally consistent across all formulations and conditions (1.22-1.24 ng/ml) with a t_{max} of 2.36-2.38 h.

Bioavailability (F) for isoniazid in all cases was 1, whilst F values for rifampicin were 0.91. This may be related to the high Fa seen with both APIs. Bioavailability for rifampicin correlates well with reported values. Rifampicin is a CYP3A4 inducer [55] and it is likely that over a longer study period (i.e. multidose over a few weeks) F would drop to around 65-70%, as a result of increased metabolism [54]. Furthermore, due to the inclusion of ascorbic acid as an antioxidant and since dissolution and degradation was not tested in simulated gastric fluid (at a lower pH), actual bioavailability values *in vivo* may differ.

Conclusion

ODTs demonstrated satisfactory performance for hardness, friability and disintegration. Dissolution profile comparison between single and FDC formulations of isoniazid indicated bioequivalence regardless of dissolution media used and this was reinforced through PBPK modelling, with no difference in pharmacokinetic parameters. Comparable bioequivalence between single and FDC was not assumed for rifampicin from dissolution comparison in FeSSIF, with drug release falling by around 15%, likely as a result of rifampicin degradation.

Clinical trial simulations reported no difference in isoniazid bioavailability between combination and single dose, despite isoniazid dissolution failing f_2 testing in FaSSIF. Additionally, no food effect was seen. Notably also, the apparent decrease in rifampicin dissolution from FDCs in FeSSIF did not result in reduced bioavailability in fed subjects, whilst FDCs in fasted subjects similarly displayed bioequivalence with the single dose formulation, highlighting a failure in f_1 and f_2 factor testing.

PBPK modelling demonstrated that the bioavailability of either drug was unaltered as a result of combination with the other in these formulations. Rapid release isoniazid and rifampicin FDC ODTs thus may be a viable and attractive formulation prospect, whilst the framework used here could be employed in the development of more complex formulations. It should be noted that the focus for these investigations was on preformulation and initial dosage form development and therefore stability studies were not carried out.

The application of PBPK modelling in this study demonstrated the ability of this technique to predict *in vivo* performance based on *in vitro* experimental work and thus overcome the difficulties in performing clinical trials in paediatric populations. Although PBPK modelling cannot replace real-world clinical testing in paediatrics, with further studies in real paediatric populations being required to confirm the results seen here, PBPK offers a powerful tool to predict efficacy, safety and bioequivalence and aid in regulatory approval.

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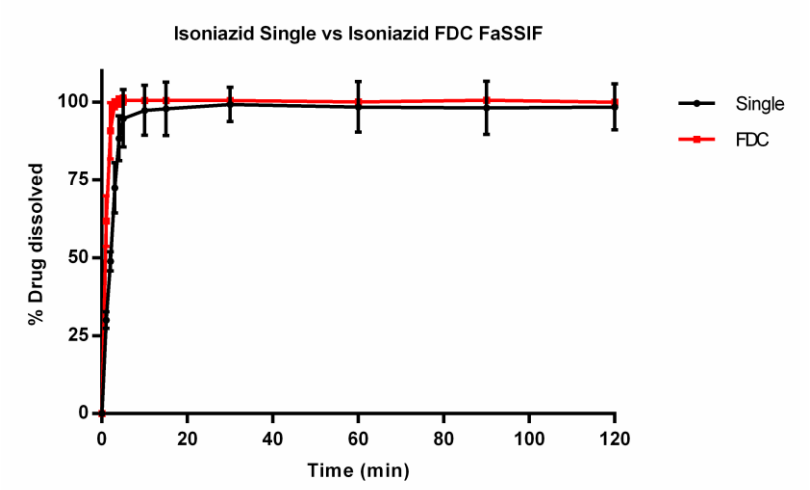

Figure 1. Isoniazid (50 mg) dissolution profiles of single and FDC formulations in fasted state biorelevant media (900 ml, 37°C) from 500 mg ODTs. Dissolution performed using USP 2 paddle apparatus (mean ± SD, n=3)

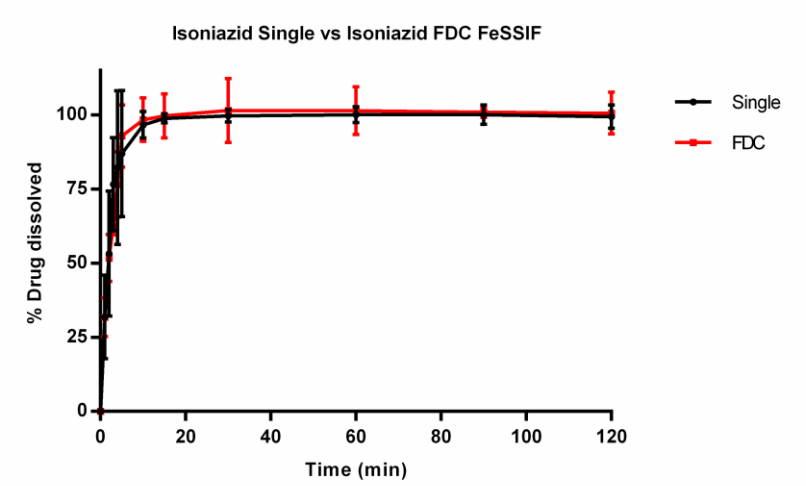

Figure 2. Isoniazid (50 mg) dissolution profiles of single and FDC formulations in fed state biorelevant media (900 ml, 37°C) from 500 mg ODTs. Dissolution performed using USP 2 paddle apparatus (mean ± SD, n=3)

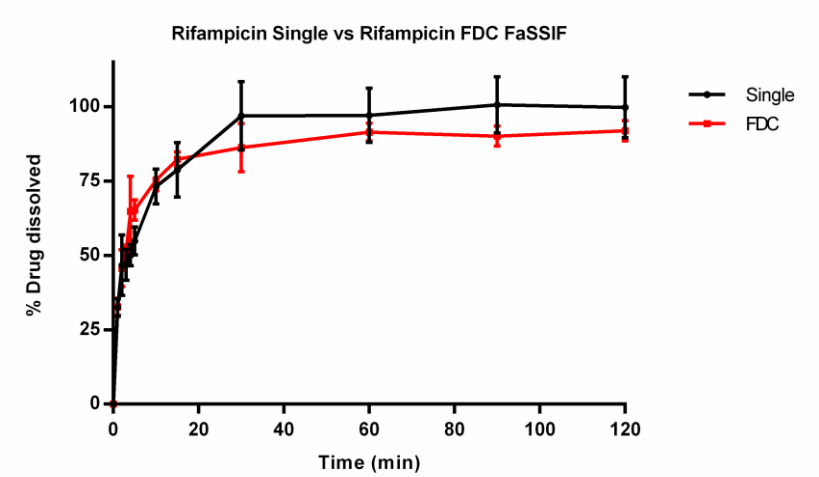

Figure 3. Rifampicin (75 mg) dissolution profiles of single and FDC formulations in fasted state biorelevant media (900 ml, 37°C) from 500 mg ODTs. Dissolution performed using USP 2 paddle apparatus (mean ± SD, n=3)

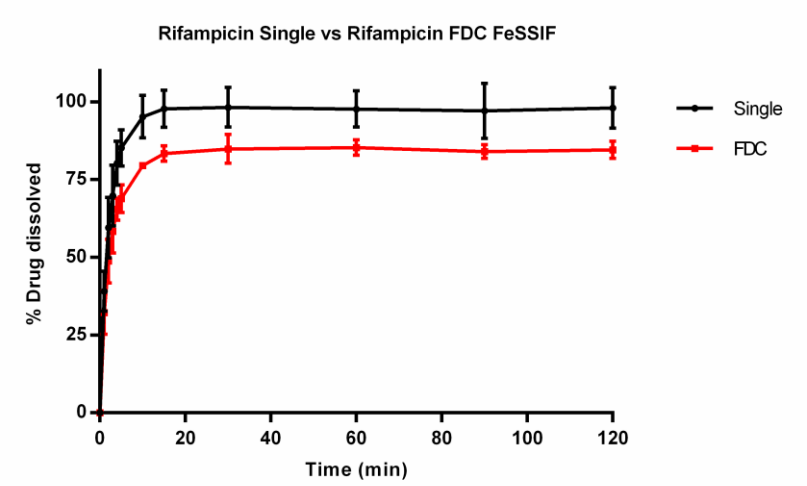

Figure 4. Rifampicin (75 mg) dissolution profiles of single and FDC formulations in fed state biorelevant media (900 ml, 37°C) from 500 mg ODTs. Dissolution performed using USP 2 paddle apparatus (mean ± SD, n=3)

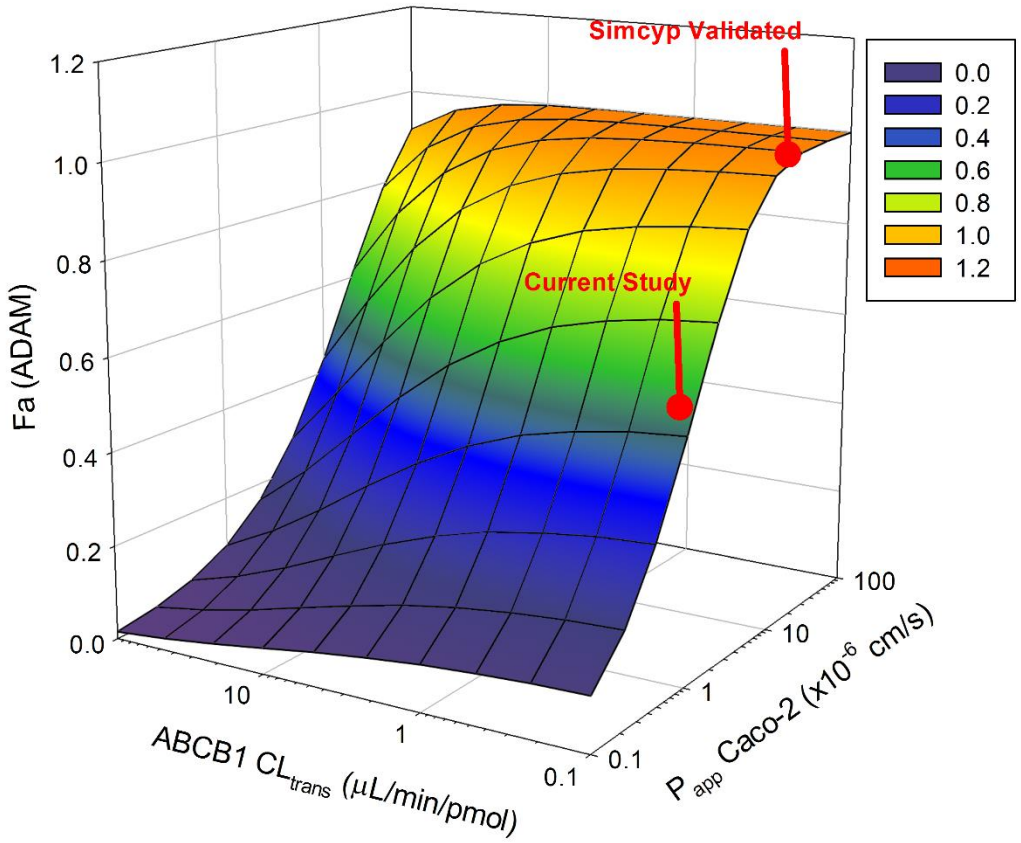

Figure 5. Sensitivity analysis of rifampicin (Simcyp default compound) fraction dose absorbed (fa) when varying P_{app} (0.1-100 x10^{-6} cm/s) and intestinal active efflux (CL_{trans}) (0.1-100 µL/min/pmol ABCB1). P_{app} values for the current study and those validated by Simcyp are highlighted.

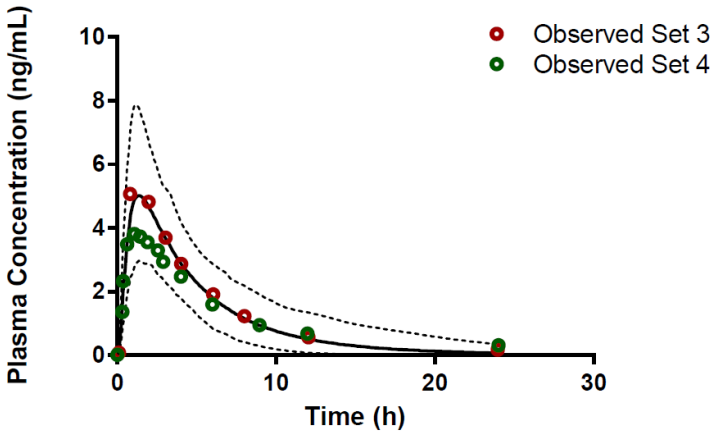

Figure 6. Simulated mean plasma profile after a 300 mg oral dose of isoniazid (solid black line). The corresponding observed data points are shown by red open circles. The grey lines represent the 5th and 95th percentiles for the predicted values. All simulations were performed using the minimal PBPK model.

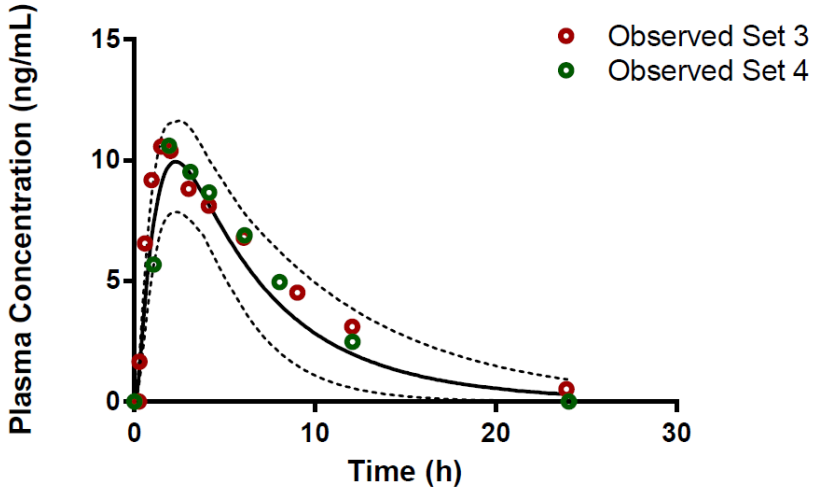

Figure 7. Simulated mean plasma profile after a 600 mg oral dose of rifampicin (solid black line). The corresponding observed data points are shown by red (set 3) or green (set 4) open circles. The grey lines represent the 5th and 95th percentiles for the predicted values. All simulations were performed using the minimal PBPK model.

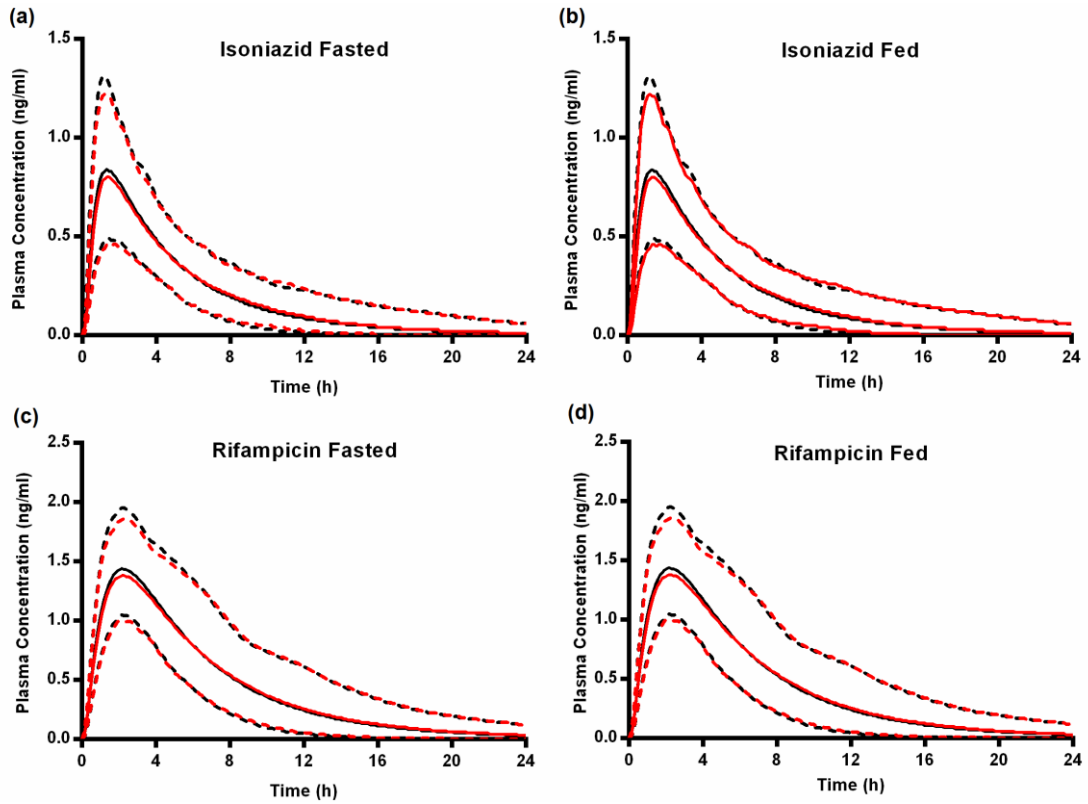

Figure 8. Simulated mean plasma profile after a 50 mg oral dose of isoniazid (a and b) and 75 mg oral dose of rifampicin (c and d) under fasted and fed conditions. Single API formulations indicated in black and fixed-dose combination in red. Solid lines represent trial mean and dashed lines represent the 5th and 95th percentiles for the predicted values.

Table 1. ODT formulations for individual dose and FDC ODTs. Values for APIs and excipients are given as % w/w for 500mg tablets. All formulations underwent compaction at 2.2 T with a 6 s dwell time.

	Isoniazid (10%)	**Rifampicin (15%)**	**Isoniazid + Rifampicin (10% + 15%)**
	F1	**F2**	**F3**
Isoniazid	50		50
Rifampicin		75	75
Pearlitol Flash	367.5	342.5	292.5
SSF (1.5%)	7.5	7.5	7.5
MCC (15%)	75	75	75

Table 2. Individual and FDC ODT properties. All formulations underwent compaction at 2.2 T with a 6 s dwell time.

	Hardness (N)	Porosity	Disintegration Time (s)	Friability (% weight loss)
F1	95.50 ± 1.15	0.26 ± 0.01	22.67 ± 1.53	1.10
F2	143.90 ± 15.47	0.25 ± 0.01	22.67 ± 1.15	0.86
F3	151.17 ± 4.48	0.23 ± 0.01	26.67 ± 2.52	0.85

Table 3. HPLC method validation for detection of isoniazid and rifampicin both alone and in combination. Data for linearity (correlation coefficient), instrument precision, accuracy (recovery), precision (% RSD), LOD and LOQ are displayed

	Instrument precision (% RSD)	Recovery (mean % ± SD)	Intraday precision (mean % RSD)	LOD (µg /ml)	LOQ (µg /ml)	Correlation coefficient
Isoniazid	0.08	99.53 ± 0.60	0.88	0.24	0.80	0.99997
Rifampicin	0.13	100.33 ± 1.13	2.07	0.14	0.46	0.99994
Isoniazid combination	0.27	99.64 ± 1.06	1.47	0.15	0.51	0.99996
Rifampicin combination	0.87	100.49 ± 1.28	1.20	0.36	1.18	0.99987

Table 4. Comparison of dissolution profiles for each compound from single and FDC formulations in FaSSIF and FeSSIF media, by difference factor f_1 and similarity factor f_2 testing. Dissolution profiles are considered similar if the f_1 value is below 15 and the f_2 value is above 50.

Compound		>85% Dissolution ≤15 min	f_1	f_2
Isoniazid	FaSSIF	Yes	14.17	32.79
	FeSSIF	Yes	3.78	65.30
Rifampicin	FaSSIF	No	9.30	55.76
	FeSSIF	No	15.55	44.82

Table 5. P_{app} values for isoniazid and rifampicin alone and in combination in A-B and B-A directions, across Caco-2 monolayers at pH 7.4 in both compartments (mean ± SD, n=3)

Compound	P_{app} 10^{-6} cm s^{-1}		Efflux Ratio
	A-B	B-A	
Isoniazid	16.37 ± 0.48	19.27 ± 0.32	1.18
Rifampicin	1.37 ± 0.12	5.95 ± 0.42	4.33
Isoniazid Combination	22.69 ± 1.21	26.98 ± 0.26	1.19
Rifampicin Combination	2.14 ± 0.19	5.58 ± 0.50	2.61

Table 6. Summary of pharmacokinetic parameters for isoniazid (50 mg) under fasted and fed conditions. Geometric mean (SD) reported for C_{max} and median (range) for AUC and t_{max}

	Isoniazid Fasted		Isoniazid Fed	
Parameters	**Single**	**Combined**	**Single**	**Combined**
AUC (ng/ml.h)	4.05 (3.14-7.10)	4.24 (3.13-7.41)	4.05 (3.14-7.10)	4.24 (3.13-7.42)
C_{max} (ng/ml)	0.74 (0.13)	0.70 (0.12)	0.74 (0.13)	0.70 (0.12)
t_{max} (h)	1.48 (1.14-1.92)	1.49 (1.21-1.96)	1.48 (1.14-1.92)	1.49 (1.12-1.91)

Table 7. Summary of pharmacokinetic parameters for rifampicin (75 mg) under fasted and fed conditions. Geometric mean (SD) reported for C_{max} and median (range) for AUC and t_{max}

Parameters	Rifampicin Fasted		Rifampicin Fed	
	Single	**Combined**	**Single**	**Combined**
AUC (ng/ml.h)	8.80 (6.63-13.63)	9.26 (6.61-13.50)	8.80 (6.63-13.63)	9.26 (6.61-13.50)
C_{max} (ng/ml)	1.24 (0.18)	1.22 (0.30)	1.24 (0.18)	1.22 (0.30)
t_{max} (h)	2.38 (1.51-2.80)	2.38 (1.80-2.85)	2.38 (1.51-2.80)	2.36 (1.80-2.86)

Table 8 Input parameter values and predicted PBPK values for simulation of pharmacokinetics of isoniazid and rifampicin.

Parameter	Isoniazid	Rifampicin
Type	Monoprotic base	Ampholyte
MW	137.1	823
LogP	-0.7	4.01
pKa	1.82	1.7,7.9
fu	0.95	0.113
Vss (L/kg)[a]	Predicted PBPK/PE	0.42 (Full PBPK)
B:P ratio	0.825	0.9
Clpo (L/min)	12	8.75
Peff (cms/s)	PE	2.15

MW: molecular weight; fu: plasma unbound fraction; Vss: steady-state volume of distribution; B:P ratio: blood-to-plasma ratio; CLpo: oral clearance; Peff: human effective permeability. [a] Vss was determined from calculation of tissue partitions coefficients within Simcyp or parameter estimated (PE).