SUPPLEMENTAL METHODS

Samples were prepared using protein precipitation with rifampicin-d3 as an internal standard for RPT and isoniazid-d4 for INH, followed by high performance liquid chromatography with tandem mass spectrometry detection on an AB Sciex API4000 instrument. Mass transitions of the protonated precursor ions m/z 877.6 and m/z 826.4 to the product ions m/z 151.1 and m/z 151.2 were monitored for RPT and rifampicin-d3, respectively. For isoniazid and isoniazid-d4, the analyte and internal standard were monitored at mass transitions of the protonated precursor ions m/z 138.1 and m/z 142.2 to the product ions m/z 79.1 and m/z 83.1,respectively. The calibration curves fitted quadratic regressions (weighted by 1/concentration^2) over the range 0.0391 to 40.0 mg/L for RPT and 0.105 to 25 μg/mL for INH. The RPT assay accuracies were between 99.4% and 106.5% with precision less than 5.1% at the limit of quantification, low, medium, and high-quality control concentrations during inter-batch validation. The accuracies for INH were between 92.2% and 107%, with precision less than 10.9% at the limit of quantification, low, medium, and high-quality control concentrations during inter-batch validation.

SUPPLEMENTAL MATERIALS

Population pharmacokinetic (PK) models for (i) rifapentine/desacetyl rifapentine and (ii) isoniazid were constructed using NONMEM (version 7.4; ICON PLC, Dublin, Ireland). Data were fitted with the first-order conditional estimation with interaction method and modeled with ordinary differential equations. The statistical significance of parameters added to nested models was determined using the likelihood ratio test (L_1/L_2); if the objective function value difference, which is described as the difference between 2 log likelihood ratios (-2LL) and follows a chi-squared distribution, between 2 models differing by a single parameter was greater than 3.84, the parameter was deemed to be significant ($p < 0.05$). Diagnostic plots (e.g., observations vs. population and individual predictions, conditional weighted residuals vs. time and population predictions, and individual profiles) were constructed to evaluate each iteration during model development. Visual predictive checks (VPC) with 1,000 simulated datasets were generated to assess model

appropriateness. Perl-speaks-NONMEM (PsN, version 4.8.1), Pirana (version 2.9.7), R (version 3.5.1), and Xpose4 (version 4.6.1) were used for data management, model development, and data visualization(1).

Rifapentine/desacetyl rifapentine. Parent plasma concentrations were characterized by a 1-compartment model with oral absorption. The absorption phase was described using three transit compartments, and a mean transit time (MTT) was estimated. HIV status (HIV-infected, HIV-uninfected) and pregnancy status (antepartum, postpartum) were tested as covariates on apparent clearance (CL/F) resulting in four CL/F estimates. Metabolite plasma concentrations were then added, and both parent and metabolite PK were modeled together assuming complete conversion from parent to metabolite (fraction metabolized (fm) equal to 1). The final parent/metabolite PK model was parameterized with CL/F, apparent volume (V/F), MTT, apparent metabolite clearance (CL_{met}/(F*fm)), and apparent metabolite volume (V_{met}/(F*fm)); oral bioavailability (F) was assumed to equal 1. Inter-individual variability (IIV) was added to CL/F, F, V/F, MTT, and CL_{met}/(F*fm). Inter-occasion variability (IOV) was tested on the absorption parameters MTT and F, but was not retained in the final model. Residual unexplained variability (RUV) was characterized by a combined additive and proportional error model. PK data that were below the lower limit of quantification (LLOQ) were excluded from the modeling analysis. Supplemental Table 1 provides the final model parameter estimates. Supplemental Figure 1 illustrates the VPCs for the parent and metabolite stratified by HIV status and pregnancy trimester.

Isoniazid. Isoniazid plasma concentrations were characterized by a 1-compartment model with oral absorption. The absorption phase was described using two transit compartments, and a MTT was estimated. A mixture model was used to estimate the proportion of the population with slow, intermediate, and fast clearance; isoniazid is metabolized by arylamine N-acetyltransferase 2 (NAT2), which is characterized by genetic polymorphisms that affect the drug's clearance (2, 3). Data were only sufficiently available to characterize two populations (slow and fast). The final PK model was parameterized with CL/F, V/F, MTT,

and the proportion of slow-acetylators in the population ($P_{slow\_acetylator}$). IIV was added to MTT and RUV was characterized by a proportional error model. PK data that were below the LLOQ were excluded from the modeling analysis. Supplemental Table 2 provides the final model parameter estimates. Supplemental Figure 2 illustrates the VPC stratified by acetylation status.

Supplemental Table 1: Parameter estimates for the rifapentine/desacetyl rifapentine pharmacokinetic model. Relative standard error (RSE), coefficient of variation (CV).

Parameter	Definition	Unit	Population estimate (RSE, %)	Inter-individual variability, CV% (RSE, %)
Rifapentine				
CL/F	Apparent clearance	-	-	9.70 (30)
[HIV negative, antepartum]	-	L/hr	1.20 (6)	-
[HIV negative, postpartum]	-	L/hr	1.53 (8)	-
[HIV positive, antepartum]	-	L/hr	1.56 (7)	-
[HIV positive, postpartum]	-	L/hr	1.60 (11)	-
V/F	Apparent volume	L	29.4 (4)	16.6 (30)
F	Bioavailability	-	1†	25.1 (11)
MTT	Mean transit time	hr	2.80 (7)	46.0 (20)
Additive error	Residual error	mcg/mL	0.326 (61)	-
Proportional error	Residual error	% CV	35.0 (11)	-
Desacetyl rifapentine				
$CL_{met}/(F*fm)$	Apparent clearance	L/hr	2.75 (7)	22.9 (17)
$V_{met}/(F*fm)$	Apparent volume	L	17.8 (7)	-
Additive error	Residual error	mcg/mL	0.0677 (60)	-
Proportional error	Residual error	% CV	42.2 (6)	-

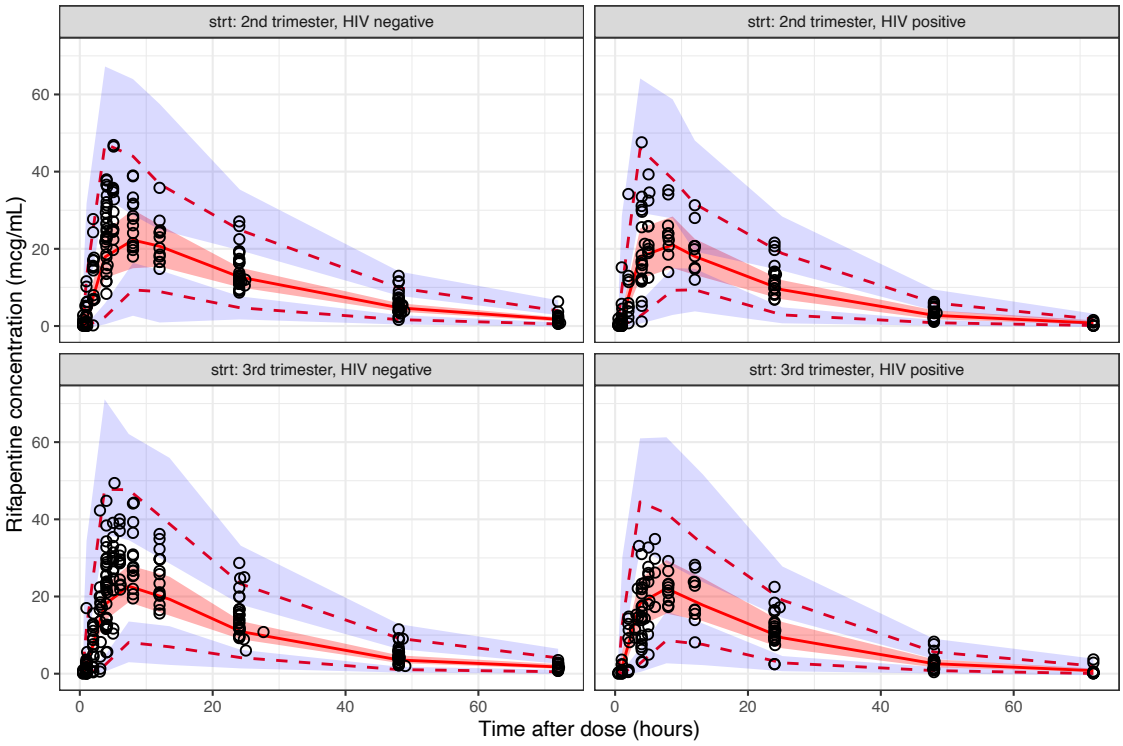
†*fixed parameter value*

Supplemental Table 2: Parameter estimates for the isoniazid pharmacokinetic model. Relative standard error (RSE), coefficient of variation (CV).

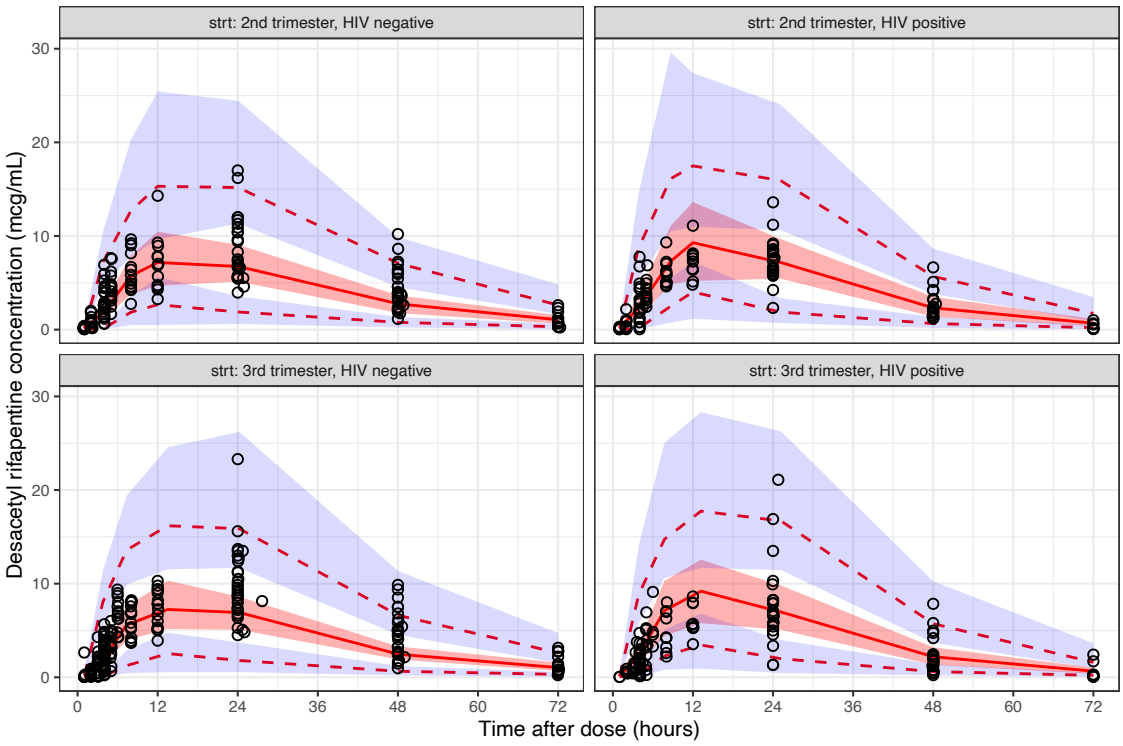
Parameter	Definition	Unit	Population estimate (RSE, %)	Inter-individual variability, CV% (RSE, %)
Isoniazid				
CL/F	Apparent clearance	-	-	-
[slow-acetylators]	-	L/hr	11.7 (12)	-
[fast-acetylators]	-	L/hr	40.8 (6)	-
V/F	Apparent volume	L	85.6 (6)	-
MTT	Mean transit time	hr	1.16 (11)	51.7 (14)
$P_{slow\_acetylator}$	Proportion of slow-acetylators	-	0.706 (10)	-
Proportional error	Residual error	% CV	37.6 (7)	-

Supplemental Figure 1: Visual predictive checks for rifapentine/desacetyl rifapentine pharmacokinetic model. VPC comparing (a) parent and (b) metabolite drug concentrations (observed data) and model predictions stratified (strt) by HIV status (HIV negative or positive) and pregnancy trimester (second or third); trimester designation is based on when the initial dose was taken. Observed data (open black circles), simulated median (solid red line), simulated 5th and 95th percentiles (dashed red lines), simulation-based 95% confidence interval (shaded regions) for median (light red), 2.5th percentile (light blue, lower), and 97.5th percentile (light blue, upper). Data deemed to be below the lower limit of quantification are omitted.

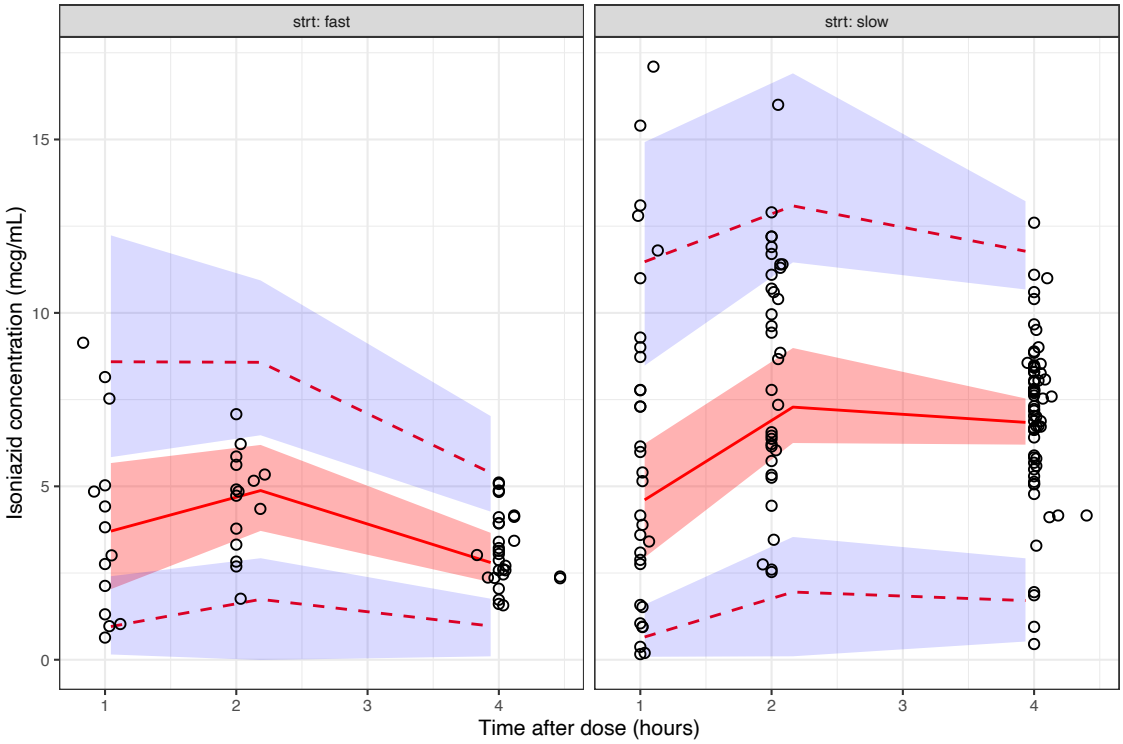
(a) Rifapentine



(b) Desacetyl rifapentine


strt: 2nd trimester, HIV negative
strt: 2nd trimester, HIV positive
strt: 3rd trimester, HIV negative
strt: 3rd trimester, HIV positive
Desacetyl rifapentine concentration (mcg/mL)
Time after dose (hours)
0
10
20
30
0
12
24
36
48
60
72

Supplemental Figure 2: Visual predictive checks for isoniazid pharmacokinetic model. VPC comparing isoniazid drug concentrations (observed data) and model predictions stratified (strt) by acetylation status (slow or fast metabolizer). Observed data (open black circles), simulated median (solid red line), simulated 5th and 95th percentiles (dashed red lines), simulation-based 95% confidence interval (shaded regions) for median (light red), 2.5th percentile (light blue, lower), and 97.5th percentile (light blue, upper). Data deemed to be below the lower limit of quantification are omitted.



Stochastic simulation-estimation methodology. The stochastic simulation-estimation (SSE) methodology was used to evaluate the sample size needed to determine key PK parameters with precision for decision making. The methodology simulates data from a planned trial with a proposed design, estimates parameters using a true and alternative model, and repeats the process at least 1000 times. The relative standard error (RSE), inter-individual variability (IIV) and PK parameter estimates (clearance, CL; volume of distribution, V; absorption rate constant, k_a) are then assessed across the iterations. From the SSE conducted for this clinical study, a sample size of 50 participants provided an RSE of 18% for the estimated contrast between median clearance in the second trimester and median clearance in the third trimester, under 10% for the other PK parameters (V, k_a), and under 20% for IIV (CL, V). These estimates of precision are considered satisfactory based on literature reviews where RSE values for these parameters are higher. Furthermore, a sample size of 50 participants was found to provide 90% power to detect one or more safety events for which the true rate of occurrence is 5 per 100 women. These statistics can be used for decision making about the risk-benefit ratio of using the regimen in pregnancy and contribute information to the safety and tolerability of the treatment. Lastly, for an interim analysis sample size of 12 and a Type 1 error rate of 1%, SSE predicted 96% power to detect an average 25% departure from mean historical clearance values.

References.

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