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Limited sampling strategies using linear regression and the Bayesian approach for therapeutic drug monitoring of moxifloxacin in tuberculosis patients

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37 **Abstract**

38

39 Therapeutic drug monitoring (TDM) of moxifloxacin is recommended to improve response to
40 tuberculosis treatment and reduce acquired drug resistance. Limited sampling strategies (LSSs) are able
41 to reduce the burden of TDM by using a small number of appropriately timed samples to estimate the
42 parameter of interest; the area under the concentration time curve. This study aimed to develop LSSs for
43 moxifloxacin alone (MFX) and together with rifampicin (MFX+RIF) in TB patients.

44 Population pharmacokinetic (popPK) models were developed for MFX (n=77) and MFX+RIF (n=24).
45 Additionally, LSSs using Bayesian approach and multiple linear regression were developed. Jackknife
46 analysis was used for internal validation of the popPK models and multiple linear regression LSSs.
47 Clinically feasible LSSs (1-3 samples; 6 h timespan post-dose; 1 h interval) were tested.

48 Moxifloxacin exposure was slightly underestimated in the one compartment models of MFX (mean -
49 5.1%, standard error [SE] 0.8%) and MFX+RIF (mean -10%, SE 2.5%). The Bayesian LSSs for MFX and
50 MFX+RIF (both 0 and 6 h) slightly underestimated drug exposure (MFX mean -4.8%, SE 1.3%; MFX+RIF
51 mean -5.5%, SE 3.1%). The multiple linear regression LSS for MFX (0 and 4 h) and MFX+RIF (1 and 6 h),
52 showed a mean overestimation of 0.2% (SE 1.3%) and 0.9% (SE 2.1%), respectively.

53 LSSs were successfully developed using the Bayesian approach (MFX and MFX+RIF; 0 and 6 h) and
54 multiple linear regression (MFX 0 and 4 h, MFX+RIF 1 and 6 h). These LSSs can be implemented in clinical
55 practice to facilitate TDM of moxifloxacin in TB patients.

Introduction

Each year, the global tuberculosis (TB) incidence declines with approximately 2%, while by 2020 an annual 4-5% decline is strived for by the World Health Organization (WHO).(1) Multidrug-resistant TB (MDR-TB) remains a major problem with an estimated number of 458,000 cases in 2017.(1) Currently, the worldwide success rate of MDR-TB treatment is 55% and this is considered low when compared to a success rate of 85% for drug-susceptible TB (DS-TB).(1)

Moxifloxacin, a fluoroquinolone, is one of the most important drugs for the treatment of MDR-TB(2), but has also been used as an alternative to first-line anti-TB drugs if not well tolerated or suggested to include in case of isoniazid resistance.(3–5) In general, the toxicity profile of moxifloxacin is rather mild, though it includes concentration dependent QTc interval prolongation and, rarely, tendinopathy.(6–9) A clinically relevant drug-drug interaction is the combination of moxifloxacin with rifampicin, since these two drugs can be used concomitantly in TB treatment. Rifampicin lowers the moxifloxacin area under the concentration-time curve of 0-24 h (AUC_{0-24}) with approximately 30% by inducing phase II metabolising enzymes (glucuronosyltransferase and sulphotransferase).(10–12)

The efficacy of fluoroquinolones is related to the ratio of AUC_{0-24} to minimal inhibitory concentration (AUC_{0-24}/MIC).(13, 14) The fluoroquinolone exposure is effective against gram-negative bacteria at an AUC_{0-24}/MIC >100-125 and against gram-positive species at an AUC_{0-24}/MIC >25-30.(13, 15, 16) An *in vitro* moxifloxacin exposure of unbound (*f*)AUC_{0-24}/MIC of >53 was able to substantially decrease the total population of *M. tuberculosis* with over 3 $\log_{10}$ CFU/ml as well as suppress emergence of drug resistance, while an *f*AUC_{0-24}/MIC >102 completely killed the fluoroquinolone sensitive population of *M. tuberculosis* without observing development of drug resistance.(17) Approximately 50% of moxifloxacin is assumed to be protein bound, although protein binding is highly variable between individuals and might be concentration dependent.(13, 16, 18, 19) Corresponding with *f*AUC_{0-24}/MIC>53 and a fraction unbound of 0.5, the target total (bound and unbound) AUC_{0-24}/MIC >100-125 is regularly used in TB, because

individual data of protein binding is often lacking.(18, 20, 21) In case of a proven susceptibility for moxifloxacin while lacking a MIC value of the strain, the target $AUC_{0\text{-}24}$ is generally set at >50-65 mg·h/L based on a critical concentration of 0.5 mg/L.(22, 23)

Therapeutic drug monitoring (TDM) is recommended by the American Thoracic Society for all second-line drugs, including moxifloxacin.(24, 25) It is important to monitor the moxifloxacin exposure in TB patients to determine an individualized dose, because of substantial inter-individual pharmacokinetic variability and relevant drug-drug interactions with the risk of treatment failure and developing drug resistance.(18, 26–28) However, routine TDM to estimate $AUC_{0\text{-}24}$ requiring frequent blood sampling is time-consuming, a burden for patients and health care professionals, and expensive. Optimising the sampling schedule by developing a limited sampling strategy (LSS) could overcome these difficulties with TDM in TB treatment.(29)

There are two main methods to develop a LSS; the Bayesian approach and multiple linear regression.(30) The advantages of the Bayesian approach are the flexible timing of samples as the population pharmacokinetic model can correct for deviations and that it takes a number of parameters into account for example sex, age, and kidney function, leading to a more accurate estimation of $AUC_{0\text{-}24}$. The advantage of multiple linear regression-based LSSs is that these do not require modelling software and $AUC_{0\text{-}24}$ can be easily estimated using only an equation and the measurement of drug concentrations. The disadvantage is that samples must be taken exactly according to the predefined schedule and the population of interest should be comparable because patient characteristics are not included in the equations to estimate drug exposure.(30)

Pranger *et al* described a LSS for moxifloxacin for the first time using t=4 and 14 h post-dose samples.(21) This sampling strategy can be considered unpractical to be used in daily practice. Magis-Escurra *et al* described LSSs to simultaneously estimate $AUC_{0\text{-}24}$ of all first-line drugs together with moxifloxacin (t=1, 4, 6 h or t=2, 4, 6 h), but did not differentiate between patients using moxifloxacin alone and

moxifloxacin in combination with rifampicin.(20) Therefore the influence of the drug-drug interaction between moxifloxacin and rifampicin, namely an increased moxifloxacin clearance, was not taken into account in these LSSs.

Therefore, the aim of this study was to develop and validate two population pharmacokinetic models of moxifloxacin (alone and with rifampicin) along with clinically feasible LSSs using the Bayesian approach as well as multiple linear regression for the purpose of TDM of moxifloxacin in TB patients.

Results

Study population

The group with moxifloxacin alone (MFX) included pharmacokinetic profiles of 77 TB patients and the group with moxifloxacin together with rifampicin (MFX+RIF) included profiles of 24 TB patients (Figure 1). The baseline characteristics sex, age and height were significantly different ($P<0.05$) between these two groups (Table 1). Additionally, the $AUC_{0\text{-}24}$ calculated with the trapezoidal rule ($AUC_{0\text{-}24,\,ref}$) was significantly lower and time of peak concentration (T_{max}) was significantly earlier in the MFX+RIF group ($P<0.05$, Table 2). Several abnormal pharmacokinetic curves (e.g. delayed absorption or single aberrant data point) were observed in both the MFX and MFX+RIF group.

Population pharmacokinetic model

For both MFX and MFX+RIF, an one compartment model with lag time resulted in the lowest Akaike Information Criterion (AIC) values and described the data best (Table 3). Two compartment models were not favourable for either MFX or MFX+RIF. A statistical comparison of the pharmacokinetic parameters of the MFX versus MFX+RIF model was provided in Table 4. Total body clearance (CL) was higher and lag time (T_{lag}) was shorter in the MFX+RIF model ($P<0.05$). Internal validation of the two models resulted in a

mean underestimation of AUC_{0-24} of 5.1% (standard error (SE) 0.8%) in the MFX model and a mean underestimation of 10% (SE 2.5%) in the MFX+RIF model (Figure 2A and Figure 3A). In the validation of the MFX model, an r^2 of 0.98, y-axis intercept of -0.3 (95% CI -1.1 to 0.5), and slope of 0.96 (95% CI 0.94-0.98) was found in the Passing Bablok regression (Figure 2B). For the MFX+RIF model, an r^2 of 0.94, y-axis intercept of -1.0 (95% CI -4.1 to 0.9), and slope of 0.98 (95% CI 0.92-1.07) was found in the Passing Bablok regression (Figure 3B).

LSS using the Bayesian approach

The best performing LSSs of MFX and MFX+RIF are shown in Table 5 and Table 6, including mean prediction error (MPE), root mean squared error (RMSE), and r^2 to evaluate the performance of the LSSs. The performance of the LSS using t=2 and 6 h samples was evaluated as well, because this strategy is currently used in many health facilities for TDM of anti-TB drugs.(31) Not all strategies met the pre-set acceptance criteria (RMSE<15%, MPE<5%).(21) Low r^2 values were observed which were caused by high interindividual variability in performance of the LSSs.

For the MFX model, an LSS using t=0 and 6 h samples was chosen for further evaluation (RSME=15.17%, MPE= 2.42%, r^2=0.874), because it required one sample less than the three-sample strategies, while RMSE was only slightly above 15%. The internal validation showed a mean underestimation of 4.8% (SE 1.3%). However, low AUC_{0-24} values were more frequently overestimated in contrast to AUC_{0-24} >40 mg*h/L mainly being underestimated by the LSS (Figure 4A). The Passing Bablok regression showed an r^2 of 0.94, y-axis intercept of 3.4 (95% CI 1.6-4.9), and slope of 0.85 (95% CI 0.80-0.91) (Figure 4B).

For the MFX+RIF model, an LSS using t=0 h and 6 h samples was chosen for further evaluation (RSME=15.81%, MPE= 2.35%, r^2=0.885), because of the benefit of requiring only 2 samples while performance in terms of RSME and MPE remained acceptable. The internal validation showed a mean

151 underestimation of 5.5% (SE 3.1%) in the Bland-Altman plot and an r^2 of 0.90, y-axis intercept of -1.3
152 (95% CI -4.4 to 2.8), and slope of 1.0 (95% CI 0.88-1.10) in the Passing Bablok regression (Figure 5).
153
154 LSS using multiple linear regression
155 Table 7 and Table **8** show the best performing LSSs for MFX and MFX+RIF. The performance of the
156 frequently used LSS using t=2 and 6 h samples was evaluated as well and included in the tables. None of
157 the MFX LSSs met the acceptance criteria (RMSE<15%, MPE<5%) as bias was above 5% for all
158 combinations. For MFX+RIF, the two three-sample strategies and LSS using t=1 and 6 h samples met the
159 acceptance criteria.
160 The MFX LSS using t=0 and 4 h samples (RSME=9.25%, MPE= 6.85%, r^2=0.957) had a comparable
161 performance to the three-sample strategies while being more clinically feasible and therefore was
162 chosen for further evaluation. In contrast to the Bayesian LSSs for MFX and MFX+RIF, a t=0 and 6 h
163 strategy was not feasible using a multiple linear regression approach as its performance was
164 substantially worse (RMSE=12.01, MPE=9.43, r^2=0.905) than the LSS using t=0 and 4 h samples. Internal
165 validation of this t=0 and 4 h LSS for MFX showed a mean overestimation of 0.2% (SE 1.3%) in the Bland-
166 Altman plot and an r^2 of 0.95, y-axis intercept of 0.1 (95% CI -2.1 to 1.6), and slope of 0.99 (95% CI 0.95-
167 1.06) in the Passing Bablok regression (Figure 6).
168 For MFX+RIF, the LSS using t=1 and 6 h samples (RSME=6.09%, MPE= 4.83%, r^2=0.971) was chosen for
169 further evaluation, because of clinical suitability in addition to good performance (RMSE<15%, MPE<5%).
170 Internal validation showed a mean overestimation of 0.9% (SE 2.1%) in the Bland-Altman plot and an r^2
171 of 0.96, y-axis intercept of -0.2 (95% CI -4.9 to 2.3), and slope of 1.02 (95% CI 0.88-1.15) in the Passing
172 Bablok regression (Figure 7).
173
174 **Discussion**

In this study, we successfully developed a population pharmacokinetic model for moxifloxacin alone and in combination with rifampicin. Furthermore, we developed and validated sampling strategies using the Bayesian approach (MFX and MFX+RIF t=0 and 6 h) and multiple linear regression (MFX t=0 and 4 h; MFX+RIF t=1 and 6 h) for both groups as well.

It was decided to develop two separate population pharmacokinetic models, and therefore also separate LSSs, for moxifloxacin alone and in combination with rifampicin after observing a significant effect of rifampicin on the pharmacokinetics of moxifloxacin. The population pharmacokinetic model of MFX+RIF showed an approximately 35% higher total body clearance of moxifloxacin when compared to the MFX pharmacokinetic model (Table 4). This was to be expected as rifampicin enhances metabolism of moxifloxacin and increases in total body clearance of 45-50% have been reported by others.(10, 32) As a result of this drug-drug interaction, pharmacokinetic profiles of MFX+RIF showed reduced moxifloxacin concentrations and 25% lower median moxifloxacin $AUC_{0\text{-}24}$ values after administration of a similar dose (Figure 1, Table 2). The latter is confirmed by a significant -17% difference in dose-corrected $AUC_{0\text{-}24,ref}$ between the MFX and MFX+RIF group (Table 2). The decrease in moxifloxacin exposure by rifampicin was estimated at 30% in previous studies (10, 12, 32), although others found non-significant or smaller decreases in moxifloxacin $AUC_{0\text{-}24}$.(21, 33) In this study we observed only a slightly smaller effect of rifampicin on the total body clearance and exposure than previously reported. This might be explained by the possibility that maximal enzyme induction was not achieved yet at the moment of sampling in a few cases, since it generally takes around 10-14 days of rifampicin treatment to reach maximal induction.(34) Furthermore, we encountered a significant, but small, difference in lag time between the MFX and MFX+RIF models and in T_{max} of the included pharmacokinetic profiles. The faster absorption of moxifloxacin in combination rifampicin was found in other studies as well, however some reported the opposite effect. This could suggest that lag time and T_{max} was not influenced by rifampicin, but more

likely by other differences between the MFX and MFX+RIF group such as concomitantly taken TB drugs or inter-individual differences in absorption due to disease state.

In addition to the population pharmacokinetic models, we developed and validated LSSs using the Bayesian approach as well as multiple linear regression for MFX and MFX+RIF. LSSs of moxifloxacin have been described before. Pranger *et al* found a Bayesian LSS with a comparable performance (RMSE=15%, MPE=-1.5%, r^2=0.90) when compared to our LSSs for MFX and MFX+RIF.(21) The LSS of Magis-Escurra *et al* performed better (RMSE=1.45%, MPE=0.58%, r^2=0.9935) than the multiple linear regression LSSs proposed in this study.(20) However, a smaller sample size (n=12) was used to establish the equation and this was not externally validated. Further, we provided suitable sampling strategies for multiple situations; in patients using moxifloxacin alone or together with rifampicin and for centres that either do or do not have pharmacokinetic modelling software available. Health care professionals may select the LSS that is the most applicable to the circumstances.

The Bayesian LSS for MFX (t=0 and 6 h) showed a slight downward trend between the bias of the estimated $AUC_{0\text{-}24}$ and the mean of the estimated and actual $AUC_{0\text{-}24}$ (Figure 4). Low $AUC_{0\text{-}24}$ values were more frequently overestimated in comparison to higher $AUC_{0\text{-}24}$ values. A possible cause might be that we could not differentiate between metabolic clearance and renal clearance in both population pharmacokinetic models due to a small range of creatinine clearance in the study population. A relatively high exposure of moxifloxacin in patients with renal insufficiency could be underestimated as renal function may be overestimated and the other way around for patients with normal renal function and relatively low exposures. The pharmacokinetic modelling software will fit a curve with the greatest likelihood of being the actual pharmacokinetic curve based on drug concentrations at 0 and 6 h together with patient characteristics and data of the entire population. However, when influence of creatinine clearance is not available the software will pick a fit with average parameters, causing overestimation in low $AUC_{0\text{-}24}$ and underestimation in high $AUC_{0\text{-}24}$ ranges. We decided not to validate one of the better

performing three-sample strategies from Table 5, since we focussed on developing a clinically feasible LSS with a strong preference for only 2 samples. Furthermore, we aimed to provide a simple and well performing alternative LSS for MFX using multiple linear regression (t=0 and 4 h). We recommend to use this LSS instead of the Bayesian LSS for MFX, particularly when low drug exposure is suspected, because overestimation of $AUC_{0\text{-}24}$ can lead to sub therapeutic dosing with treatment failure and acquired drug resistance as possible harmful consequence.(26, 36, 37)

In this study we decided to validate one LSS for each situation (Bayesian or multiple linear regression; MFX or MFX+RIF), due to the significant influence of rifampicin on the pharmacokinetics of moxifloxacin and so there would be a suitable LSS for every patient in each health care centre. The LSSs using multiple linear regression performed rather well in our study population, but is less flexible in patients with different characteristics. A Bayesian LSS is therefore preferred for patients who are not comparable to our study populations as the population pharmacokinetic model is able to include some patient characteristics. Clinicians are guided to the best option for TDM of moxifloxacin by following the decision tree in Figure 8. For implementation of moxifloxacin TDM using LSSs in daily practice, it would be convenient to be able to use one sampling strategy for both MFX and MFX+RIF. This study showed that it is possible to use t=0 and 6 h samples in a Bayesian LSS for both MFX as well as MFX+RIF and probably even in a multiple linear regression LSS for MFX+RIF after successful validation. Unfortunately, a multiple linear regression strategy for MFX alone using t=0 and 6 h samples was not feasible because of inferior performance. Considering that TB patients are treated with a combination of multiple anti-TB drugs, one single LSS suitable for all drugs of interest is the ideal situation, but unfortunately also rather challenging due to the various pharmacokinetic properties of the different drugs. Others did succeed in developing a LSS using multiple linear regression for simultaneously estimating exposure of all first-line drugs and moxifloxacin in a small population of TB patients.(20) A 2 and 6 h post-dose sampling strategy is frequently used for TDM of anti-TB drugs as it is believed to be able to estimate C_{max} as well as to detect

246 delayed absorption.(31) However, better performances were found for the LSSs proposed in this study,
247 although the 2 and 6 h LSS performed within acceptable limits as well in the Bayesian approach and the
248 multiple linear regression.
249 In general, we noticed large inter-individual pharmacokinetic variation in terms of moxifloxacin
250 concentrations (Figure 1), C_{max}, and AUC_{0-24} (Table 2) as described earlier,(18) but also in K_a and CL/F
251 (Table 4). Patients received 400, 600, or 800 mg moxifloxacin; this obviously influenced drug
252 concentration, C_{max}, and AUC_{0-24}, but not all variation could be explained by different dosage regimes. For
253 MFX, AUC_{0-24} corrected to a 400 mg standard dose was ranged from 10.2 to 79.1 mg*h/L and for
254 MFX+RIF a range of 10.0 to 47.4 mg*h/L. This substantial inter-individual variation is the reason why
255 TDM of moxifloxacin is helpful to assure optimal drug exposure and thus minimize the risk of treatment
256 failure and developing acquired drug resistance.(26, 27) The estimated AUC_{0-24} using one of the LSS
257 proposed together with the MIC of the *M. tuberculosis* strain will provide valuable information on the
258 optimal moxifloxacin dose to be used in an individual patient.
259 A limitation to the study is the exclusion of the creatinine clearance from the population
260 pharmacokinetic model. As discussed earlier, this could have led to the observed bias in the MFX LSS
261 using 0 and 6 h samples as approximately 20% of moxifloxacin is eliminated unchanged in the urine. On
262 the contrary, a well performing LSS using multiple linear regression (t=0 and 4 h) is a suitable alternative
263 for MFX. The lack of prospective or external validation of the population pharmacokinetic model and
264 LSSs could be considered as another limitation. However, we were able to collect a large dataset to
265 develop the model and clinically feasible LSSs using a sufficient number of pharmacokinetic profiles. A
266 strength of our study was that a large part of our dataset consisted of drug concentrations which were
267 collected as part of daily routine TDM. During visual check of the data we noticed several abnormal
268 curves (both MFX and MFX+RIF) that for instance showed delayed absorption with T_{max} values of 4-6 h.
269 These curves were not excluded from the study. The models and LSSs appeared to be able to adapt to

this delayed absorption. In most cases, the subsequent decision to either increase the dose or not was similar. For these reasons, we expect the results as reported in this study to represent the clinical practice of TDM using these LSSs very closely. The small sample size of the MFX+RIF group can be considered as a limitation as well, although comparable to previously published LSS studies.(21, 38–41) We consider this sample size as sufficient for exploratory objectives, since this is the first study that developed separate LSSs for moxifloxacin alone and in combination with rifampicin. Future research can build on the results described in this study.

In conclusion, we developed and validated two separate pharmacokinetic models for moxifloxacin alone and in combination with rifampicin in TB patients. We provided data to show significant differences in drug clearance and drug exposure between these groups. Furthermore, we developed and validated LSS based on the Bayesian approach (MFX and MFX+RIF 0 and 6 h) and multiple linear regression (MFX 0 and 4 h; MFX+RIF 1 and 6 h) that can be used to perform TDM on moxifloxacin in TB patients.

Materials and methods

Study population

This study used three databases. Database 1 consisted of retrospective data of routine TDM in 67 tuberculosis patients treated at Tuberculosis Center Beatrixoord, University Medical Center Groningen, The Netherlands and was collected between January 2006 and May 2017, partly published earlier.(18) All patients received moxifloxacin (with or without rifampicin) as part of their daily TB treatment and pharmacokinetic curves were obtained as part of routine TDM care. Each patient was only included once. Varying sampling schedules were used, but most profiles included t=0, and 1, 2, 3, 4, and 8 h post-dose samples. Pharmacokinetic profiles consisting of less than 3 data points were excluded. The second database included data of 25 TB patients participating in a clinical study in Thessaloniki, Greece.(33) After at least 12 days of treatment with moxifloxacin with or without rifampicin, blood samples were

collected at t=0, and 1, 1.5, 2, 3, 4, 6, 9, 12, and 24 h after drug intake. The third database consisted of pharmacokinetic data of 9 Brazilian TB patients receiving 400 mg moxifloxacin (no rifampicin) daily in an early bactericidal activity study.(14) At the fifth day, blood samples were collected at t=0, and 1, 2, 4, 8, 12, 18 and 24 h after drug intake.

As steady state is reached within 3-5 days of treatment with moxifloxacin, all data was collected during steady state conditions.(11) In general, no informed consent was required, due to the retrospective nature of the study.

The total study population was split in two groups; patients that received moxifloxacin alone (MFX) and patients that received moxifloxacin together with rifampicin (MFX+RIF), because of the pharmacokinetic drug-drug interaction between rifampicin and moxifloxacin.(10) As sample collection in the MFX+RIF group was performed after a median number of days on rifampicin treatment of 35 (IQR 13-87), maximum enzyme induction by rifampicin was expected to be reached in most patients.(35)

Patient characteristics of both groups were tested for significant differences, median (interquartile range (IQR)) using the Mann-Whitney U test and n (%) using the Fisher's exact test in IBM SPS Statistics (23, IBM Corp., Armonk, NY). P values <0.05 were considered significant.

Population pharmacokinetic model

For each group, MFX and MFX+RIF, a population pharmacokinetic model was developed using the iterative two-stage Bayesian procedure of the KinPop module of MWPharm (version 3.82, Mediware, The Netherlands). As the pharmacokinetics of moxifloxacin have been described with one compartment (14, 21) as well as two-compartment models (42, 43), both types were evaluated. The population pharmacokinetic parameters of the models were assumed to be log normally distributed with a residual error and concentration dependent standard deviation (SD=0.1+0.1*C, where C is the moxifloxacin concentration in mg/L). Because the bioavailability (F) of moxifloxacin is almost complete (11) and

pharmacokinetic data following intravenous administration was not available, F was fixed at 1 in the analysis and pharmacokinetic parameters are presented relative to F. Moxifloxacin is mainly metabolised in the liver by glucuronosyltransferase and sulfotransferase (approximately 80%).(11) Only total body clearance (CL), the sum of metabolic and renal clearance, was included in the model development, because it was not possible to determine renal clearance due to a small range of creatinine clearance values in our dataset.

We started the analysis with a single default one compartment model for both MFX and MFX+RIF developed by Pranger *et al* using a very similar methodology.(21) This study found comparable pharmacokinetic parameters of MFX and MFX+RIF, although likely due to a small sample size. Two default two compartment models were used, one for MFX and one for MFX+RIF.(42, 44) Modelling was started with all parameters fixed and Akaike Information Criterion (AIC) was used to evaluate the model.(45) Subsequently, one by one parameters were Bayesian estimated and each step was evaluated by calculation of the AIC. A reduction of the AIC with at least 3 points was regarded as a significant improvement of the model.(46) One compartment models included the parameters CL, volume of distribution (V), and absorption rate constant (K_a). Two compartment models included the parameters K_a, CL, inter-compartmental clearance (CL_{12}), central volume of distribution (V_1), volume of distribution of the second compartment (V_2), and lag time for absorption (T_{lag}). Afterwards, T_{lag} was added to the best performing one compartment model and evaluated for goodness of fit as well, because of oral intake of moxifloxacin. The default two compartment models already included T_{lag}. The final models of MFX and MFX+RIF were chosen based on AIC values.

The final models were internally validated using 11 different (n-7) sub models for MFX and 12 (n-2) sub models for MFX+RIF, each leaving out randomly chosen pharmacokinetic curves. All pharmacokinetic curves were excluded once (jackknife analysis). The Bayesian fitted $AUC_{0\text{-}24}$ of each left out curve ($AUC_{0\text{-}24,\ fit}$) was compared with the $AUC_{0\text{-}24}$ calculated with the trapezoidal rule ($AUC_{0\text{-}24,\ ref}$) using a Bland-Altman

plot and Passing Bablok regression (Analyse-it 4.81, Analyse-it Software Ltd, Leeds, United Kingdom). In the calculation of $AUC_{0\text{-}24,\ ref}$, moxifloxacin concentrations at t=0 and 24 h after drug intake were assumed to be equal due to steady state conditions. C_{max} (mg/L) was defined as the highest observed moxifloxacin concentration and T_{max} (h) as the time at which C_{max} occurred. Non-compartmental parameters ($AUC_{0\text{-}24,\ ref}$, dose-corrected $AUC_{0\text{-}24,\ ref}$ to the standard dose of 400 mg, C_{max}, T_{max}) and population pharmacokinetic model parameters of the MFX and MFX+RIF group were compared and tested for significant differences using the Mann-Whitney U test.

LSS using Bayesian approach

Using the Bayesian approach, we performed two separate analyses to develop LSSs; one for MFX and one for MFX+RIF. Using Monte Carlo simulation in MWPharm, 1000 virtual pharmacokinetic profiles were created to represent the pharmacokinetic data used in the development of the LSS. The reference patient for the Monte Carlo simulation was selected based on representative pharmacokinetic data and patient characteristics. For MFX, a 36 year old male with a bodyweight of 57 kg, height of 1.60 m, BMI of 22.2 kg/m^2, serum creatinine of 74 µmol/L, and moxifloxacin dose of 7.0 mg/kg was chosen. For MFX+RIF, a 56 year old male with a bodyweight of 56 kg, height of 1.63 m, BMI of 21.1 kg/m^2, serum creatinine of 80 µmol/L, and moxifloxacin dose of 7.1 mg/kg was selected. The LSSs were optimised using the steady state $AUC_{0\text{-}24}$. Only clinically feasible LSSs using 1-3 samples between 0 and 6 h post-dose and sample interval of 1 h were tested. The LSSs were evaluated using acceptance criteria for precision and bias (RMSE<15%, MPE<5%).(18) For both MFX and MFX+RIF, one LSS was chosen for internal validation based on performance as well as clinical feasibility. The $AUC_{0\text{-}24}$ estimated with the chosen LSS ($AUC_{0\text{-}24,\ est}$) was compared with $AUC_{0\text{-}24,\ ref}$ using a Bland-Altman plot and Passing Bablok regression. Additionally, the performance of a LSS using 2 and 6 h post-dose samples was evaluated, because this is a LSS frequently used for TDM of anti-TB drugs.(31)

366

367 LSS using multiple linear regression

368 Two separate analyses (MFX and MFX+RIF) using multiple linear regression were performed.

369 Only clinically suitable LSSs (1-3 samples, 0-6 h post-dose, sample interval 1 h) were included in the

370 analysis. Each analysis excluded the pharmacokinetic curves without data at the selected time points of

371 the LSS, resulting in a variable number of included curves (N). Multiple linear regression in Microsoft

372 Office Excel 2010 was used to evaluate the correlation of moxifloxacin concentrations at the chosen time

373 points of the LSS and $AUC_{0\text{-}24,\ ref}$. The acceptance criteria (RMSE<15%, MPE<5%) were applied to each

374 LSS.(18) Internal validation using 11 different (n-6) sub analyses for MFX and 14 (n-1) sub analyses for

375 MFX+RIF was used to evaluate the performance of the LSSs. Each sub analysis excluded randomly chosen

376 profiles and all profiles were excluded once (jackknife analysis). Agreement of $AUC_{0\text{-}24,\ est}$ and $AUC_{0\text{-}24,\ ref}$

377 was tested using a Bland-Altman plot and Passing Bablok regression.

378

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512

513 Table 1. Patient characteristics of the study population. Data is presented as median (IQR) unless
514 otherwise stated.

Parameter	MFX n=77	MFX+RIF n=24	P value
Male sex [n(%)]	47 (61.0)	21 (87.5)	0.023[a]
Age (yr)	33 (25-41)	48 (36-62)	<0.001[b]
Ht (m)	1.65 (1.59-1.74)	1.72 (1.64-1.76)	0.047[b]
Wt (kg)	58.0 (52.5-68.2)	55.5 (52.3-63.9)	0.500[b]
Dose (mg/kg bodywt)	7.0 (5.9-8.1)	7.3 (6.4-7.7)	0.629[b]
BMI (kg/m^2)	21.2 (19.3-23.5)	20.1 (17.6-22.7)	0.053[b]
Serum creatinine (µmol/L)	71 (59-83)	73 (63-91)	0.752[b]
Number of samples per curve	7 (6-8)	10 (7-10)	<0.001[b]
Days on rifampicin treatment at time of sampling	NA	35 (13-87)	NA

515 [a] Fisher exact test

516 [b] Mann-Whitney U test

517

518 Table 2. Non-compartmental parameters ($AUC_{0\text{-}24,\ ref}$, dose corrected $AUC_{0\text{-}24,\ ref}$ to 400 mg standard dose,

519 C_{max}, and T_{max}) of MFX and MFX+RIF, presented as median (IQR).

Parameter	MFX (n=77)	MFX+RIF (n=24)	P-value
$AUC_{0\text{-}24,\ ref}$ (mg·h/L)	34.0 (25.2-49.2)	25.5 (20.4-31.6)	0.006[a]
Dose corrected $AUC_{0\text{-}24,\ ref}$ (mg·h/L, per 400 mg)	30.8 (24.7-40.3)	25.5 (19.1-31.3)	0.014[a]
C_{max} (mg/L)	3.00 (2.27-4.64)	2.83 (2.25-3.90)	0.407[a]
T_{max} (h)	2 (1-3)	1.5 (1-2)	0.018[a]

520 [a] Mann-Whitney U test

521 Table 3. Starting parameters of the default one compartment and two compartment models of MFX and
522 MFX+RIF together with the parameters of the final models based on AIC.

Parameter	Default model MFX	Final model MFX	Default model MFX+RIF	Final model MFX+RIF
One compartment				
CL (L/h)	18.500±8.600	14.655±5.683	18.500±8.600	19.898±8.800
V_d (L/kg bodyweight)	3.000±0.7000	2.7467±1.0077	3.000±0.7000	2.8264±0.6902
K_a (/h)	1.1500±1.1600	6.2904±4.8164	1.1500±1.1600	7.3755±6.8205
T_{lag} (h)	NA	0.8769±0.2357	NA	0.7460±0.1093
AIC	5564	903	1361	236
Two compartments				
CL (L/h)	11.800±0.740	13.428±5.494	49.100±2.550	18.108±8.570
CL_{12} (L/h)	5.620±1.080	5.620±1.080	3.150±0.800	3.150±0.800
V_1 (L/kg bodyweight)	2.5300±0.0800	2.4898±1.0838	2.8400±0.1500	2.7004±0.7535

V_2 (L/kg bodyweight)	0.6900±0.1300	0.6900±0.1300	0.8900±0.1900	0.8900±0.1900
K_a (/h)	16.7000±2.9200	3.2774±2.9422	2.3200±0.5600	6.2314±9.0508
T_{lag} (h)	0.4600±0.0800	0.7940±0.3720	0.6000±0.0700	0.7312±0.1995
AIC	11892	940	2995	249

523

524 Table 4. Comparison of pharmacokinetic parameters of the population pharmacokinetic model of MFX

525 versus MFX+RIF. Geometric mean±SD.

Parameter	MFX (n=77)	MFX+RIF (n=24)	P value
CL/F (L/h)	14.655±5.683	19.898±8.800	0.004[a]
V_d/F (L/kg bodyweight)	2.7467±1.0077	2.8264±0.6902	0.534[a]
K_a (/h)	6.2904±4.8164	7.3755±6.8205	0.231[a]
T_{lag} (h)	0.8769±0.2357	0.7460±0.1093	<0.001[a]

526 [a] Mann-Whitney U test

527

528 Table 5. LSSs of moxifloxacin without RIF using the Bayesian approach, including MPE, RMSE, and r^2.

Sampling time point (h)			MPE (%)	RMSE (%)	r^2
5			2.69	24.64	0.659
6			1.74	22.00	0.726
2	6		-2.20	20.83	0.742
0	5		2.84	15.82	0.864
0	6		2.42	15.17	0.874
0	4	6	0.97	13.22	0.883

0	5	6	1.03	12.97	0.888

529

530 Table 6. LSSs of moxifloxacin with RIF using the Bayesian approach, including MPE, RMSE, and r^2.

Sampling time point (h)			MPE (%)	RMSE (%)	r^2
5			-1.97	22.35	0.768
6			-0.79	19.22	0.826
2	6		-2.89	18.38	0.832
0	5		1.88	16.67	0.877
0	6		2.35	15.81	0.885
0	4	6	1.06	14.10	0.907
0	5	6	0.79	13.73	0.912

531

532 Table 7. LSSs of moxifloxacin without RIF using linear regression, including the equation to calculate

533 $AUC_{0\text{-}24,\ est}$, number of included curves (N), MPE, RMSE, and r^2.

Sampling time point (h)			Equation[a]	N	MPE (%)	RMSE (%)	r^2
4			$AUC_{0\text{-}24,\ est}$= 3.47+12.32*C4	66	12.68	17.02	0.862
6			$AUC_{0\text{-}24,\ est}$ = 2.27+15.01*C6	22	14.85	16.89	0.822
2	6		$AUC_{0\text{-}24,\ est}$ = -1.44+3.55*C2+11.24*C6	22	10.02	12.27	0.901
0	3		$AUC_{0\text{-}24,\ est}$ = 3.61+28.67*C0+5.38*C3	53	10.08	13.36	0.917
0	4		$AUC_{0\text{-}24,\ est}$ = 1.10+20.76*C0+8.68*C4	66	6.85	9.42	0.957

0	2	4	$AUC_{0\text{-}24,\ est}$ = 1.10+20.37*C0+0.92*C2+7.71*C4	65	6.91	9.25	0.958
0	1	4	$AUC_{0\text{-}24,\ est}$ = 1.00+21.06*C0+0.66*C1+8.02*C4	63	7.07	9.23	0.958

[a] C0, C1, etc., are moxifloxacin concentrations at t=0 h, t=1 h, etc.

Table 8. LSSs of MFX+RIF using multiple linear regression, including the equation to calculate $AUC_{0\text{-}24,\ est}$, number of included curves (N), MPE, RMSE, and r^2.

Sampling time point (h)			Equation[a]	N	MPE (%)	RMSE (%)	r^2
3			$AUC_{0\text{-}24,\ est}$ =-2.76+13.28*C3	18	8.27	11.10	0.907
6			$AUC_{0\text{-}24,\ est}$ = 0.95+16.44*C6	16	6.93	8.87	0.941
2	6		$AUC_{0\text{-}24,\ est}$ = 0.08+1.21*C2+15.02*C6	13	6.23	7.88	0.945
0	6		$AUC_{0\text{-}24,\ est}$ = 1.38+7.40*C0+14.05*C6	16	5.85	6.99	0.960
1	6		$AUC_{0\text{-}24,\ est}$ = 1.43+0.22*C1+16.25*C6	14	4.83	6.09	0.971
0	3	6	$AUC_{0\text{-}24,\ est}$ = 1.20+10.66*C0-0.39*C3+13.52*C6	15	4.85	5.31	0.977
0	2	6	$AUC_{0\text{-}24,\ est}$ = 0.46+9.99*C0+0.13*C2+13.39*C6	13	4.20	4.66	0.978

[a] C0, C1, etc., are moxifloxacin concentrations at t=0 h, t=1 h, etc.

539 Figure 1. Moxifloxacin concentrations of the pharmacokinetic curves of MFX (n=77) and MFX+RIF (n=24)

540

541 Figure 2. Bland-Altman plot (A) and Passing Bablok regression (B) of internal validation (n-7) of

542 population pharmacokinetic model of MFX (n=77).

543

544 Figure 3. Bland-Altman plot (A) and Passing Bablok regression (B) of internal validation (n-2) of

545 population pharmacokinetic model of MFX+RIF (n=24).

546

547 Figure 4. Bland-Altman plot (A) and Passing Bablok regression (B) of internal validation of Bayesian LSS

548 (t=0 and 6 h) of MFX (n=77).

549

550 Figure 5. Bland-Altman plot (A) and Passing Bablok regression (B) of internal validation of Bayesian LSS

551 (t=0 and 6 h) of MFX+RIF (n=24).

552

553 Figure 6. Bland-Altman plot (A) and Passing Bablok regression (B) of internal validation (n-6) of LSS using

554 multiple linear regression (t=0 and 4 h) of MFX (n=66).

555

556 Figure 7. Bland-Altman plot (A) and Passing Bablok regression (B) of internal validation (n-1) of LSS using

557 multiple linear regression (t=1 and 6 h) of MFX+RIF (n=14).

558

559 Figure 8. Clinical guide for choosing the best LSS for TDM of moxifloxacin alone or in combination with
560 rifampicin.

561