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New approach to rifampicin stability and first-line anti-tubercular drug pharmacokinetics by UPLC-MS/MS

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ABSTRACT

Successful tuberculosis (TB) therapy requires achieving sufficient exposure to multiple drugs. Limited stability of several first-line anti-TB drugs might compromise reliable therapeutic drug monitoring (TDM). We developed and validated a sensitive and selective UPLC-MS/MS method for simultaneous quantification of isoniazid (INH), pyrazinamide (PZA), rifampicin (RIF), its metabolite 25-desacetylrifampicin and degradation products: rifampicin quinone and 3-formyl-rifampicin. Analysis was completed from a very small plasma volume (20 μL) using only protein precipitation with methanol. Chromatographic separation was achieved on a Kinetex Polar C18 column (2.6 μm; 150 × 3 mm) with a mobile phase consisting of 5 mM ammonium acetate and acetonitrile, both containing 0.1 % formic acid, in gradient elution. The analytes were detected using a positive ionization mode by multiple reaction monitoring. The LLOQ for RIF and its degradation products was 0.1 μg/mL, 0.05 μg/mL for INH, and 0.2 μg/mL for PZA. The method was validated based on the FDA guidance. The application of the method was confirmed in the analysis of RIF, INH, and PZA, as well as RIF metabolism/degradation products in plasma samples of patients with TB. Based on the detailed stability study of the analyzed compounds at various storage conditions, we proposed recommendations for handling the plasma and serum samples in TDM and other pharmacokinetic studies.

1. Introduction

Global Tuberculosis Report 2022 published by the World Health Organization (WHO) (https://www.who.int/publications/i/item/9789240061729) estimated that about 25 % of the world population is infected with tuberculosis (TB) bacterium. Among the infected people, 5 – 10 % will develop active TB disease in their lifetime. In 2021, there were 10 million people who developed TB disease, and about 64 % of them were diagnosed. Although the disease is preventable and curable, there were approximately 1.6 million TB-related deaths in 2021, among them 1.4 million in HIV-negative people and 0.2 million in HIV-positive people.

According to Centers for Disease Control and Prevention document on Tuberculosis (TB) - Treatment for TB Disease (https://www.cdc.gov/tb/topic/treatment/tbdisease.htm), currently, ten drugs are approved by the FDA for treating drug-susceptible TB. The preferred regimen for treating TB caused by organisms that are suspected to be drug resistant consists of a 2-month intensive phase therapy with four anti-TB drugs: rifampicin (RIF), isoniazid (INH), pyrazinamide (PZA), and ethambutol (EMB) followed by a 4-month continuation phase with INH and RIF. However, when the drug-susceptibility test results showed that the TB bacilli are fully susceptible, EMB can be avoided.

The rationale for therapeutic drug monitoring (TDM) of TB drugs is well documented [1–4]. TDM improves the safety and efficacy of the treatment in TB patients with co-morbidities and co-medications, e.g., HIV, renal or hepatic insufficiency [4,5]. The most commonly

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recommended therapeutic ranges for plasma or serum peak (2 h post-dose) concentrations are: 8 – 24 μg/mL for RIF, 3 – 6 μg/mL for INH, 20 – 60 μg/mL for PZA, and 2 – 6 μg/mL for EMB [1–4,6]. *Mota* et al. [7] found no strong association between serum drug concentrations of the first-line anti-TB agents and treatment outcomes. On the contrary, *Sileshi* et al. [6] showed such associations. *Märtson* et al. [5] suggested that TDM based on PK/PD indexes (e.g., AUC/MIC) and implementing optimal sampling strategy could be a game changer in dose adjustments. Despite decades of using first-line anti-TB drugs, further studies and unified guidance are required for optimal TDM [5,6].

Implementing TDM for TB drugs in routine clinical practice requires understanding the limited stability of anti-TB agents. INH is unstable at room temperature (RT) in whole blood, serum, and plasma; therefore, the samples should be frozen as soon as possible after collection [2].

Rifampicin quinone (RIF-Q), a main derivative of RIF, is a product of nonenzymatic autooxidation [8,9]. Presence of RIF-Q is considered an impurity in the quality control of the RIF tablet production [9]. Data on RIF-Q in the analysis of human clinical samples are scarce in the context of potential *in vivo* RIF – RIF-Q interconversion and sample stability assessment. Some authors indicated that clinical samples should be treated with antioxidants, e.g., vitamin C, to prevent RIF autooxidation during storage and handling [10,11]. *Peloquin* [12] reported no benefits from such an additive; however, blank plasma was significantly processed before adding RIF, namely charcoal stripped, ultracentrifuged, and filtered. *Mishra* et al. [8] emphasized the need for strictly regulated storage conditions for samples containing RIF as oxidation to RIF-Q significantly depends on temperature. Recently, some anti-microbial activity of RIF-Q was reported, which might be partially attributed to interconversion of RIF and RIF-Q in the presence of microbes [9]. The authors emphasized that RIF-Q should be considered in analyzing microbial resistance mechanisms to RIF in its derivative presence.

Decomposition of RIF is pH dependent. The main degradation product of RIF at low pH is 3-formyl-rifampicin (3-F-RIF) (Supplementary Fig. A.1), a poorly soluble compound with high anti-microbial activity *in vitro* but inactive *in vivo*. The formation of 3-F-RIF in the stomach's acidic environment is more pronounced in the presence of INH and can contribute to reduced bioavailability of RIF [13]. At alkaline pH, RIF undergoes deacetylation to 25-desacetylrifampicin (25-D-RIF). The mild alkaline conditions also favor the formation of RIF-Q [14].

LC-MS/MS methods for quantifying various anti-TB agents in human samples have been used in TDM, as summarized by *Kuhlin* et al. [15]. Some methods were designed to measure the first-line anti-TB drugs [16–25], others also included their metabolites [10,26–29]. The assay by Kim et al. quantifies 20 anti-TB drugs in human plasma in two separate runs, which require a different sample preparation procedure [30]. Among the cited papers focusing on the first-line anti-TB agents, very few mention RIF-Q [10,19,28]. Kivrane et al. [28] reported RIF as a sum of masses – 821.4 (specific for RIF-Q) and 823.4 (specific for RIF). Pršo et al. [19] emphasized the possible RIF autooxidation and therefore excluded RIF from their assay due to suspected inaccuracy. Xing et al. [10] prevented sample autooxidation by adding vitamin C. Majority of publications did not mention any additives to prevent RIF autooxidation in real samples [16–18,20–24,26,29,30], or did not demonstrate applicability of the developed assay in the analysis of clinical samples [27].

In this study, we developed and validated the UPLC-MS/MS method useful for TDM of first-line anti-tubercular agents: INH, PZA, and RIF. Additionally, it allows detailed analysis of RIF stability and/or metabolism by quantifying 25-D-RIF, 3-F-RIF, and RIF-Q. The method requires a very low volume of plasma (20 μL), with fast and straightforward sample preparation (protein precipitation). The application of the method was confirmed in the analysis of clinical samples. In addition to our stability analysis, we comprehensively reviewed available literature and proposed recommendations for sample handing in TDM or other pharmacokinetic studies.

2. Material and methods

2.1. Standards and reagents

Reference standards of RIF (>97 % purity), INH (≥99 % purity), and PZA (certified reference material) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Reference standards of RIF-Q (95.19 % purity), 3-F-RIF (96.42 % purity), and 25-D-RIF (95.06 % purity), as well as internal standards: rifampicin-D_3 (RIF-IS; 98.15 % purity), and pyrazinamide-$^{15}N,D_3$ (PZA-IS; 99.5 % purity) were supplied by Toronto Research Chemicals (North York, ON, Canada). Acetonitrile, water, and methanol were purchased from Sigma-Aldrich (St. Louis, MO, USA), while isopropanol from Fisher Chemical (Fair Lawn, NJ, USA). All solvents were of LCMS grade. Mobile phase additives (LCMS grade) were formic acid (LCMS grade, >99.0 % purity) and ammonium formate (LCMS grade, ≥99 % purity). They were supplied by Fisher Chemical, and Honeywell (Morristown, NJ, USA), respectively. Blank human plasma of individual donors for method validation was received from BioIVT (Westbury, NY, USA).

2.2. Instrumentation and chromatographic conditions

Chromatographic separation was carried out on an ExionLC AD system (AB Sciex, Framingham, MA, USA) equipped with a Kinetex Polar C18 column (2.6 μm; 150 × 3 mm), protected by a UHPLC C18 (3.0 mm ID) guard column (Phenomenex, Torrance, CA, USA). Other Phenomenex columns were also tested during method development: Synergi Polar-RP (50 × 2 mm, 2.5 μm), Synergi Fusion–RP (50 × 2 mm, 4 μm), Kinetex Phenyl-Hexyl (50 × 2.6 mm, 4.6 μm), Kinetex C8 (50 × 3 mm, 2.6 μm). The column oven and the autosampler were maintained at 40 °C and 15 °C, respectively. The injection volume was 4 μL.

Mobile phase A (MPA) consisted of 5 mM ammonium formate in water containing 0.1% formic acid, while mobile phase B (MPB) was acetonitrile containing 0.1 % formic acid. The mobile phase was pumped at 0.35 mL/min flow rate, with the following gradient conditions: 0 min – 99:1 (MPA:MPB, v/v), 8 min – 1:99, 10 min – 1:99, 10.5 min – 99:1, 12 min – 99:1. A mixture of deionized MilliQ water (Millipore, Billerica, MA, USA) and acetonitrile (50:50, v/v) containing 0.1 % formic acid was used for external and internal needle rinsing. The mobile phase was directed to waste between 0.1 and 1.0 min, and after 9.5 min of the run to minimize mass spectrometer contamination. The total run time was 12 min

Due to a significant carry-over of INH, an additional autosampler and column cleaning method was applied. MPA and MPB were of the same composition but were pumped with W-shaped gradient conditions (flow rate of 0.35 mL/min): 0 min – 99:1 (MPA:MPB, v/v), 1 min – 99:1, 2 min – 1:99, 3 min – 1:99, 4 min – 99:1, 5 min – 1:99, 6 min – 1:99, 7 min – 99:1, 8 min – 1:99, 9 min – 1:99, 10 min – 99:1, 12 min – 99:1. Furthermore, a mixture of isopropanol and water (4 μL, 50:50, v/v) had to be injected every 4–5 calibrators or samples to prevent significant INH carry-over.

2.3. Mass spectrometer settings

Detection was accomplished with an AB Sciex QTRAP 6500 + mass spectrometer (Framingham, MA, USA) equipped with an IonDrive Turbo V Source and operated in positive ionization mode (ESI+) using multiple reaction monitoring modes (MRM). The ion source parameters were: source temperature, 400 °C; ion spray voltage, 5 kV; collision gas (nitrogen), medium level; ion source gas 1 and 2 (zero air), 60 psi and 40 psi, respectively; curtain gas (nitrogen), 30 psi. Nitrogen and zero air were provided by Genius 1024 generator (Peak Scientific; Billerica, MA, USA). The monitored transitions and compound-specific parameters are listed in Table 1. Dwell time for each transition was 150 ms.

Table 1
MRM settings for all analyzed compounds and internal standards. Transitions used for quantification are marked in bold.

Analyte	Precursor ion (*m/z*)	Fragment ion (*m/z*)	Collision Energy (V)
RIF	824.3	792.2	25
		398.7	37
RIF-Q	822.2	**790.2**	25
		397.6	37
3-F-RIF	727.2	**667.1**	17
		641.2	71
25-D-RIF	782.3	**750.2**	17
		399.7	33
INH	138.0	**121.0**	19
		79.0	39
PZA	124.0	64.0	7
		81.0	23
RIF-IS	827.3	**795.3**	25
		151.1	37
PZA-IS	128.0	**84.0**	25
		99.9	9

Abbreviations: rifampicin (RIF), rifampicin quinone (RIF-Q), 3-formylrifampicin (3-F-RIF), 25-desacetylrifampicin (25-D-RIF), isoniazid (INH), and pyrazinamide (PZA), rifampicin-D3 (RIF-IS), and pyrazinamide-15 N,D3 (PZA-IS).

2.4. Preparation of stock solutions and standard solutions

Stock solutions of RIF, RIF-Q, 25-D-RIF, INH, PZA, and PZA-IS at concentrations of 1 mg/mL, 3-F-RIF of 2.5 mg/mL and RIF-IS of 0.5 mg/mL were prepared by dissolving the appropriate amount of a compound in methanol.

The standard solutions were prepared by diluting the stock solutions with methanol to receive the following concentrations: 1.0, 2.5, 5.0, 10.0, 25.0, 50.0, 100.0, 200.0 and 300 μg/mL RIF; 1.0, 2.5, 5.0, 10.0, 25.0, 50.0, 100.0 and 200.0 μg/mL RIF-Q, 3-F-RIF and 25-D-RIF; 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, 50.0 and 100.0 μg/mL INH; 2.0, 4.0, 10.0, 20.0, 40.0, 100.0, 200.0 and 400.0 μg/mL PZA; 0.5 μg/mL RIF-IS and 5 μg/mL PZA-IS. All solutions were prepared in amber glass vials and stored at − 20 °C.

2.5. Preparation of calibrators and quality control samples

The standard solutions were diluted with blank plasma (10-fold, v/v) to obtain the calibrators at concentrations of 0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 20.0, and 30 μg/mL RIF; 0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0 and 20.0 μg/mL RIF-Q, 3-F-RIF and 25-D-RIF; 0.05, 0.1, 0.25, 0.5, 1.0, 2.5, 5.0 and 10.0 μg/mL INH; 0.2, 0.4, 1.0, 2.0, 4.0, 10.0, 20.0 and 40.0 μg/mL PZA; 0.05 μg/mL RIF-IS and 0.5 μg/mL PZA-IS. Since RIF-Q was present in the RIF standard solutions, two series of calibration samples were prepared: the first one containing RIF only and the second one with a mixture of all other analytes. Quality control (QC) samples were prepared at the lower limit of quantification (LLOQ), low, medium, and high concentrations (Table 2).

For protein precipitation, an aliquot of 20 μL of plasma calibrators and QCS was mixed with 100 μL of freshly prepared internal standards solution in cold (5 °C) methanol (0.5 μg/mL RIF-IS and 5 μg/mL PZA-IS), shaken out vigorously for 10 min, and centrifuged at 15,700× g for 10 min at 5 °C. Then, 20 μL of the supernatant was transferred to a glass vial with 100 μL of cold (5 °C) acetonitrile with 0.1 % of FA, and vortexed. To improve stability, the samples were kept at 5 °C before transferring to the autosampler.

2.6. Validation

Validation of the method was accomplished according to the U.S. Food and Drug Administration (FDA) guidance (https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf).

Table 2
Intra-day and inter-day precision and accuracy of the analytes.

Compound	QC level	concn (μg/mL)	Precision (%RSD)		Accuracy (%)	
			Intra-day n = 5	Inter-day n = 3	Intra-day n = 5	Inter-day n = 3
RIF	LLOQ	0.100	12.5	12.3	94.4	115
	Low	0.250	5.19	11.6	91.5	95.2
	Medium	2.50	10.9	4.31	99.6	107
	High	30.0	10.5	4.96	94.0	98.6
RIF-Q	LLOQ	0.100	3.64	1.02	94.7	98.0
	Low	0.250	6.22	4.27	100	99.4
	Medium	2.50	7.21	11.6	114	111
	High	20.0	4.17	1.58	100	87.7
3-F-RIF	LLOQ	0.100	6.05	10.1	104	106
	Low	0.250	10.7	9.03	106	97.2
	Medium	2.50	3.35	5.55	110	107
	High	20.0	10.1	0.850	110	91.7
25-D-RIF	LLOQ	0.100	8.48	3.84	102	105
	Low	0.250	10.5	1.44	96.1	98.2
	Medium	2.50	13.9	6.03	93.7	108
	High	20.0	11.4	10.9	90.1	100
INH	LLOQ	0.050	5.92	7.21	86.0	100
	Low	0.100	4.39	10.4	90.2	106
	Medium	1.00	4.28	4.25	90.0	95.9
	High	10.0	5.90	1.10	94.4	103
PZA	LLOQ	0.200	10.1	7.47	83.2	107
	Low	0.400	2.40	4.26	87.3	98.8
	Medium	4.00	2.71	9.10	94.3	98.4
	High	40.0	2.00	4.26	102	103

Abbreviations: rifampicin (RIF), rifampicin quinone (RIF-Q), 3-formylrifampicin (3-F-RIF), 25-desacetylrifampicin (25-D-RIF), isoniazid (INH), and pyrazinamide (PZA).

2.6.1. Selectivity

Method selectivity was evaluated by analyzing the chromatograms of blank plasma samples from six individual sources with the sample containing IS and analytes at the LLOQ level to check the potential interferences at the retention times.

2.6.2. Calibration curves

Calibration curves were created for the ratio of the peak area of the analytes to that of the IS as a function of the analyte concentration covering the range of 0.1–30.0 μg/mL for RIF, 0.1–20.0 μg/mL for RIF-Q, 3-F-RIF and 25-D-RIF, 0.05–10.0 μg/mL for INH and 0.2–40.0 μg/mL for PZA in plasma. RIF-IS was used as an internal standard for RIF, RIF-Q, 3-F-RIF, and 25-D-RIF, while PZA-IS was for PZA and INH. Each calibration curve was analyzed using linear and non-linear regression with an appropriate weighting factor ($1/x$ or $1/x^2$).

2.6.3. LLOQ, precision, and accuracy

LLOQ was defined as the lowest concentration of RIF, RIF-Q, 3-F-RIF, 25-D-RIF, INH, and PZA determined by the method within the relative standard deviation (RSD) and relative error (RE) not exceeding 20 %. The LLOQ samples with plasma concentrations of 0.1 μg/mL for RIF, RIF-Q, 3-F-RIF, and 25-D-RIF, 0.05 μg/mL for INH, and 0.2 μg/mL for PZA were prepared and determined.

Intra- and inter-day accuracy and precision were evaluated by analyzing QCs at LLOQ, low, medium and high concentrations in six replicates on the same day and over three consecutive days. The RSD between the nominal and the measured concentrations should be within ± 15 % for QCs and ± 20 % for the LLOQ.

2.6.4. Carry-over

Carry-over was evaluated by injecting the blank sample immediately after injection of the calibrator at the highest concentration of the analytes. The signal of the blank sample should not exceed 20 % of the analyte signal at the LLOQ level.

2.6.5. Matrix effect

The effect of co-eluting matrix components on ionization was evaluated for each analyte and internal standards using six individual plasma samples spiked with QCs at low and high concentrations of the analytes. Matrix factor (MF) was calculated by dividing the peak measured in a blank matrix spiked with analytes after protein precipitation by the peak area of the analytes at equivalent concentrations in the absence of the matrix. Moreover, the IS normalized MF was calculated by dividing the analyte's MF by the IS's MF. The RSD of the IS-normalized MF should not be greater than 15 %.

2.6.6. Stability

Stability of the analytes in plasma samples was assessed at low and high QCs concentration levels in three replicates under different storage conditions. Freeze–thaw stability was determined after three cycles of the plasma samples freezing at − 20 °C and thawing at room temperature (RT). For short-term stability, samples were stored on a benchtop for 2 h and 4 h at RT. In addition, the stability in plasma samples was evaluated for 24 h and 7 days storage at − 20 °C. Stability in processed samples was also assessed after storage at 15 °C in autosampler for 12 h. In addition, the stability of stock solutions and working solutions was investigated after storage at − 20 °C for 1 month.

2.6.7. In vivo application

Application of the validated method has been demonstrated in quantifying RIF, RIF-Q, 25-D-RIF, INH, and PZA in plasma samples from adults (n = 5) undergoing treatment for active TB disease with first-line RIF-containing regimen (intensive and continuation phase of anti-TB therapy). Participants were recruited from New Jersey (Rutgers Global TB Institute's Lattimore Practice and Middlesex, Hudson, and Patterson County clinics) and Virginia (University of Virginia and surrounding Virginia Department of Health clinics). Subjects were excluded if they were incontinent, anuric, pregnant, or breastfeeding. They signed the informed consent to participate in the study. Blood samples were drawn 2 h after the administration of the drugs. Human subjects' approval was obtained through Rutgers Health Sciences IRB Pro2018001857 and University of Virginia Health Sciences IRB HSR #20944.

3. Results

3.1. LC-MS/MS analysis

Since the analyzed TB drugs differ in polarity, we evaluated several analytical columns and mobile phase compositions with different additives (formic acid, ammonium formate 5–15 mM) to obtain the best chromatographic separation of the analytes in a reasonable time. INH and PZA are highly hydrophilic compounds; according to DrugBank (go.drugbank.com), the experimental logP values are –0.7 for INH and –0.6 for PZA, while logP of RIF is 2.7. Therefore, we evaluated columns with stationary phases designed to increase the retention of polar compounds while ensuring hydrophobic selectivity. Additionally, similarity in the chemical structure of RIF and its quinone and the presence of both RIF and RIF-Q in pure standards of their counterparts required a complete chromatographic separation of the compounds. On the Synergi Polar-RP column, designed for polar and aromatic compounds, both INH and PZA had the same retention times of 0.5 min; the RIF peak was not separated from RIF-Q (Supplementary Fig. A.2A). The Kinetex C8 column with the less hydrophobic alkyl chains should enhance retention of polar constitutes. However, the results were no better, with overlapping peaks of INH and PZA as well as RIF and RIF-Q (Supplementary Fig. A.2B). To improve the separation of the compounds, we tried to use the Kinetex Phenyl-Hexyl column indicated for resolving aromatic hydrocarbons. However, the peak shapes of RIF, RIF-Q, and 25-D-RIF were very poor (Supplementary Fig. A 0.2C). Application of the Synergi Fusion–RP column that offers balanced polar and hydrophobic selectivity achieved sufficient retention of PZA of 1.5 min and separate RIF from RIF-Q. However, both INH and PZA yielded poor peak shapes (Supplementary Fig. A.2D). Optimal separation of all compounds was achieved using Kinetex Polar C18 column and gradient elution, and the total time of analysis was 12 min (Supplementary Fig. A.2E).

3.2. Method validation

3.2.1. Selectivity

Detection of RIF, RIF-Q, 3-F-RIF, 25-D-RIF, INH, and PZA using MRM mode was highly selective, with no interferences from endogenous compounds at the expected retention time of the analytes in blank plasma samples obtained from six individual donors. When comparing the zero plasma samples to the QCs at the LLOQ level of the analytes, it was found that PZA-IS is contaminated with PZA, but the signal intensity of the analyte in zero sample comprised < 20% of that at the LLOQ level. Representative MRM chromatograms of the blank plasma sample, calibration standard containing analytes at LLOQ level, and a sample obtained from a patient are presented in Fig. 1.

3.2.2. Calibration curves

Calibration curves were analyzed at eight-point levels for 3-F-RIF, 25-D-RIF, RIF-Q, INH, and PZA, and at nine-point levels for RIF. The concentration ranges of the TB drugs were established based on expected plasma concentrations in patients under a standard dose regimen. Linearity of analytical response in calibration ranges was confirmed for RIF, 25-D-RIF, INH, and PZA when weighting factors $1/x$ (for INH) or $1/x^2$ (for RIF, 25-D-RIF, and PZA) were applied. A power function best fitted RIF-Q and 3-F-RIF.

3.2.3. LLOQ, precision and accuracy

The LLOQ for the analytes was estimated at the lowest calibration standard concentration, where the %RSD and the accuracy were 1.0–12.5 % and 83.2 – 115.0 %, respectively. The intraday precision and accuracy for the analytes prepared at low, medium, and high levels in six replicates each were in the ranges 2.0–13.9 % and 86.0 – 114.0 %, respectively. Based on the results from three consecutive days, the inter-day precision and accuracy estimated at low, medium, and high levels ranged 0.85–11.6 % and 87.7–111 %, respectively. The respective %RSD and accuracies for all analytes are summarized in Table 2.

3.2.4. Carry-over

Injection of the blank sample following the calibrator at the highest concentration of the analytes revealed a significant carry-over for INH. Several gradients and injections of blank samples at various configurations were tested to eliminate this effect. Implementing the cleaning procedure described in Section 2.2 allowed reducing the carry-over to 0.2–3.3 %.

3.2.5. Matrix effect

As shown in Table 3, matrix components did not significantly suppress or enhance the MS signal of the analytes, as proved by the IS-normalized MF ranging from 0.92 to 1.15. However, the effect on the 25-D-RIF analytical signal may depend on the matrix source. The %RSD of the parameter calculated at low and high concentrations of 25-D-RIF from six different plasma sources was 21.6 % and 18.3 %, respectively.

3.2.6. Stability

Results of the stability under different conditions are listed in Table 4. All the analytes were stable in plasma samples at RT when stored for 1 h (accuracy: 85.2 – 108 %). When stored at RT for 4 h, a significant reduction in INH low and high concentration was observed (accuracy: 69.2 % and 78.0 %, respectively). Moreover, 3-F-RIF and 25-D-RIF decayed at high levels by more than 15 %, while RIF-Q concentrations increased by 17–18 %. At the same time, RIF concentrations decreased by 9–12 %. Although the values are less than the 15% threshold, they may suggest a partial decomposition of RIF to RIF-Q. All

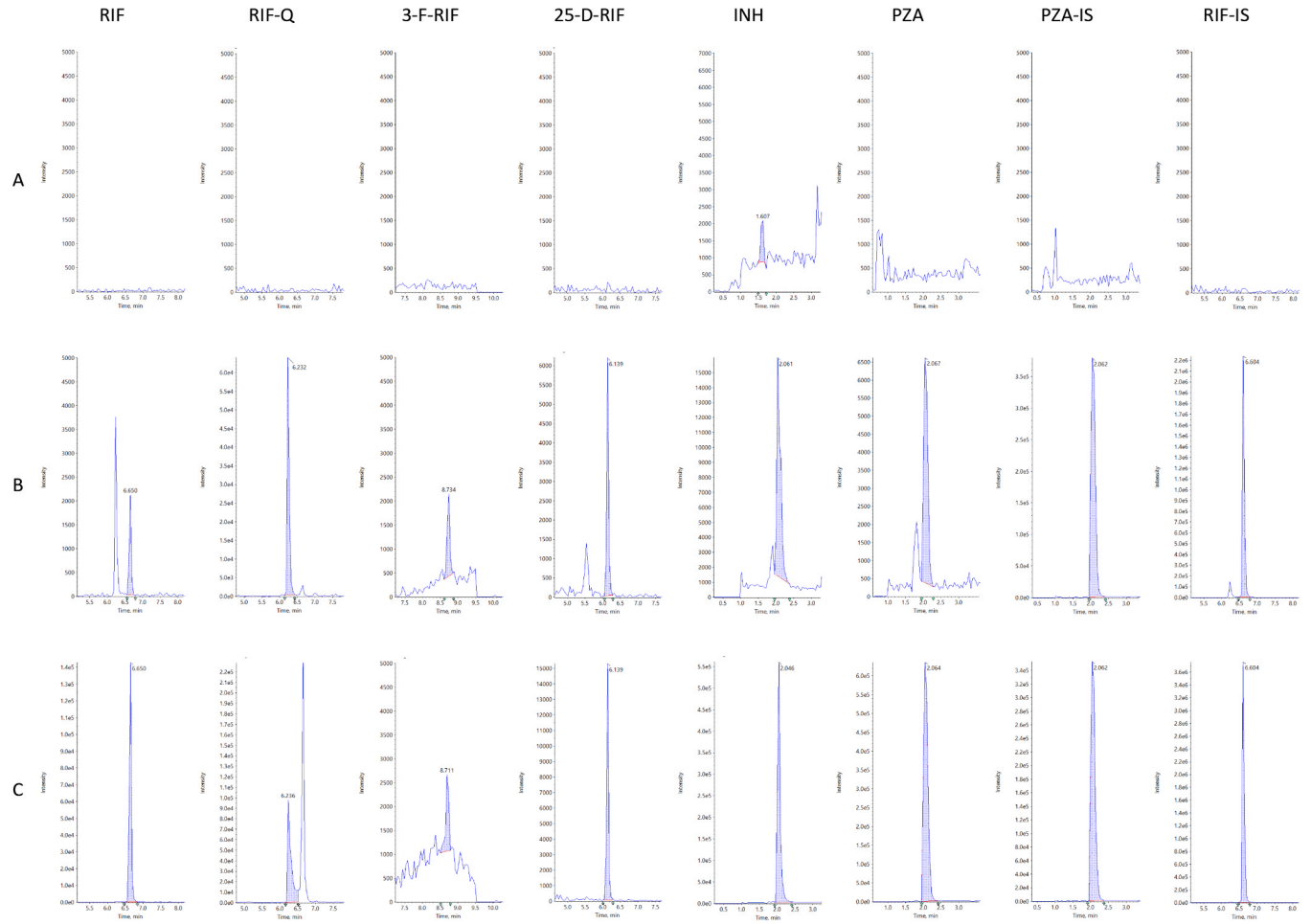

Fig. 1. Representative MRM chromatograms for the analysis of rifampicin (RIF), rifampicin quinone (RIF-Q), 3-formylrifampicin (3-F-RIF), 25-desacetylrifampicin (25-D-RIF), isoniazid (INH), and pyrazinamide (PZA) in human plasma; internal standards are rifampicin-D_3 (RIF-IS), and pyrazinamide-$^{15}N,D_3$ (PZA-IS). Ion chromatograms represent: A) unspiked blank human plasma; B) blank human plasma spiked at the LLOQ level (0.1 μg/mL for RIF, RIF-Q, 3-F-RIF, and 25-D-RIF; 0.05 μg/mL for INH; 0.2 μg/mL for PZA), internal standards at levels 0.05 μg/mL for RIF-IS and 0.5 μg/mL for PZA-IS; C) human plasma from the patient undergoing treatment for active TB disease with first-line RIF-containing regimen (measured concentrations: 5.101 μg/mL for RIF, 0.139 μg/mL for RIF-Q, <LLOQ for 3-F-RIF, 0.143 μg/mL for 25-D-RIF, 5.289 μg/mL for INH, 28.683 μg/mL for PZA).

Table 3
Matrix effect expressed by IS-normalized matrix factor.

Compound	QC level	concn [μg/mL]	IS-normalized MF mean ± SD (n = 6)	% RSD
RIF	Low	0.25	0.95 ± 0.08	8.50
	High	30	0.93 ± 0.04	4.13
RIF-Q	Low	0.25	1.15 ± 0.12	10.6
	High	20	0.94 ± 0.10	10.7
3-F-RIF	Low	0.25	0.93 ± 0.13	14.4
	High	20	0.92 ± 0.09	10.0
25-D-RIF	Low	0.25	0.97 ± 0.21	21.6
	High	20	0.96 ± 0.18	18.3
INH	Low	0.10	1.03 ± 0.04	3.89
	High	10	1.13 ± 0.08	7.56
PZA	Low	0.40	1.10 ± 0.05	4.78
	High	40	0.93 ± 0.04	4.06

Abbreviations: rifampicin (RIF), rifampicin quinone (RIF-Q), 3-formylrifampicin (3-F-RIF), 25-desacetylrifampicin (25-D-RIF), isoniazid (INH), and pyrazinamide (PZA).

tested compounds were found stable when stored at − 20 °C for 24 h; a significant decrease in RIF, RIF-Q, and 25-D-RIF was noticed after 7 days of storage at − 20 °C. After three cycles of freezing at − 20 °C and thawing at RT, RIF, 3-F-RIF, 25-D-RIF, and INH concentrations decreased by more than 15 %. Only 25-D-RIF at a high concentration was unstable in the autosampler when stored for 12 h. All analytes and IS stock solutions remained unchanged for 1 month at − 20 °C.

3.2.7. *In vivo application*

Plasma concentrations of RIF, INH, and PZA, as well as RIF metabolites and degradation products, were measured using the established UPLC-MS/MS method in the samples taken from 5 patients at 2 h after drug administration. The mean concentrations of the analytes are provided in Table 5. The measured concentrations of all analytes except 3-F-RIF were within the calibration ranges of the method.

4. Discussion

Due to the high prevalence of TB worldwide, especially in Asian countries, there has been an increasing demand for a suitable method for analyzing anti-TB drugs in biological fluids. Simple UV–VIS detection was applied in analytical methods due to relatively high concentrations of anti-TB drugs in the body [31]. However, the absorption spectra of RIF overlaps with the spectra of its degradation products, 3-F-RIF and 25-D-RIF (Supplementary Fig. A.3), which may lead to an overestimation of the drug concentrations. Therefore, for analysis of anti-TB drugs, the LC-MS methods are widely employed as they offer the molecular specificity to distinguish between the drugs and their metabolites [10,16,26–29]. However, the main limitations of these studies are

Table 4
Stability of the analytes in plasma samples.

Compound	QC level	Stability (accuracy, %)					
		Stored at RT		Stored at − 20 °C		Freeze-thaw (3 cycles, −20 °C)	Autosampler (12 h, 15 °C)
		1 h	4 h	24 h	7 days		
RIF	Low	88.0	88.0	90.4	76.8	71.8	86.6
	High	108	90.6	93.9	80.7	89.8	87.7
RIF-Q	Low	104	117	110	82.9	109	109
	High	107	118	108	83.9	96.0	118
3-F-RIF	Low	108	110	96.8	104.3	81.5	98.8
	High	98.5	84.9	91.8	88.0	78.6	109
25-D-RIF	Low	85.9	91.2	90.3	84.0	83.6	91.6
	High	99.5	82.5	93.1	94.8	89.9	82.8
INH	Low	87.0	69.2	95.6	87.7	53.7	105
	High	87.2	78.0	96.1	115	74.3	106
PZA	Low	85.3	92.5	85.8	93.5	85.8	105
	High	85.2	113	95.0	112	86.1	112

Abbreviations: rifampicin (RIF), rifampicin quinone (RIF-Q), 3-formylrifampicin (3-F-RIF), 25-desacetylrifampicin (25-D-RIF), isoniazid (INH), and pyrazinamide (PZA).

Table 5
Concentrations of TB drugs and RIF metabolites in patients' plasma samples (n = 5) at 2 h after administration.

Compound	concn (μg/mL) (mean ± SD)
RIF	6.59 ± 3.33
RIF-Q	0.192 ± 0.082
3-F-RIF	< LLOQ
25-D-RIF	0.281 ± 0.313
INH	3.17 ± 1.74
PZA	34.0 ± 8.93

Abbreviations: rifampicin (RIF), rifampicin quinone (RIF-Q), 3-formylrifampicin (3-F-RIF), 25-desacetylrifampicin (25-D-RIF), isoniazid (INH), and pyrazinamide (PZA).

inadequate separation of the analytes, large sample volume required for the analysis, time-consuming sample pretreatment, and limited stability assessment.

The established UPLC-MS/MS method allowed us to obtain an excellent chromatographic separation of RIF from its degradation products and enabled accurate quantification of all the analytes, including RIF-Q. The presence of RIF-Q was observed in clinical samples by other authors [19,28], but its concentration has not been determined so far.

For the isolation of anti-TB drugs of different polarity from plasma samples, various methods, requiring from 50 μL [28,29] to even 500 μL [27] of plasma, were applied based mainly on liquid-liquid extraction [16], solid phase extraction [19] and protein precipitation [10,17,18,20–24,27–30]. In our method, protein precipitation with methanol allowed us to reduce the amount of a sample to 20 μL of plasma, similar to *Hee* et al. using combined procedures [26].

The method was successfully validated based on FDA guidance regarding selectivity, linearity and LLOQ, precision and accuracy, matrix effect, carry-over, and stability under different conditions. The current LLOQ values for RIF, 25-D-RIF, INH, and PZA are lower than previously reported [10,18,19,21,23,25,27,28,30]. Other authors estimated lower LLOQ for RIF, INH, and PZA but used larger plasma volumes (100 μL) [20,24]. *Sundell* et al. [16] reported lower LLOQ for 25-D-RIF of 0.04 μg/mL, but the LLOQ values for other compounds were higher than in the present method. Literature data regarding the LLOQ of RIF-Q and 3-F-RIF in plasma samples and other validation parameters are unavailable. The observed matrix effect was insignificant, although it varied for 25-D-RIF depending on the plasma source. *Hee* et al. mentioned additional sample cleaning to remove phospholipids which might suppress the MS signal and negatively affect the precision and accuracy [26]. Nevertheless, the observed validation parameters for 25-D-RIF, including linearity, accuracy, and precision of the analyte, were within the recommended ranges; thus, we can assume that matrix components do not affect the analyte quantitation significantly. Other authors reported the influence of matrix source on the results of MF determination for RIF [21], EMB, INH, and INH metabolite - isonicotinic acid [28].

Individualized drug dosing guided by drug plasma concentrations could be of value in patients with TB. Blood sampling can be limited to one or two time points. Commonly, a sample 2 h post-dose is taken to capture the peak concentrations [32]. Application of the present UPLC-MS/MS method for plasma analysis of the studied compounds revealed a subtherapeutic RIF concentration in some patients (6.59 ± 3.33 μg/mL, range 2.42 – 11.0 μg/mL). INH and PZA average values of 3.17 ± 1.74 μg/mL and 34.0 ± 8.93 μg/mL were within the therapeutic ranges. However, subtherapeutic levels of INH were noticed in individual subjects (range 0.98 – 5.29 μg/mL). Although the group of patients was small and served to confirm the applicability of the developed method, the results obtained are consistent with previous findings. Plasma concentrations of RIF and INH below the therapeutic ranges were reported by Kivrane et al. [28] and Xing et al. [10], while Song et al. [29] noticed a high inter-patient variability of anti-TB drug levels at 2 h after drug intake at standard dosing.

The mean plasma concentration of 25-D-RIF in the present study was 0.281 ± 0.313 μg/mL and was higher than the value 0.05 ± 0.13 μg/mL obtained by Kivrane et al. [28] at the same sampling time. Although 25-D-RIF exhibits anti-microbial activity and its concentrations can be important for accurate drug monitoring, recommended ranges for the compound were not established. Vanbrabant et al. [33] suggested measuring the serum levels of the sum of RIF and 25-D-RIF 3 h after taking the drug. Still, there is no uniform criterion for the target value. According to Song et al. [29], the 25-D-RIF/RIF ratio could be a valuable parameter for determining drug metabolic status. The reported metabolic ratio values were in the range of 0.015 – 0.065 (average 0.032 ± 0.011), similar to the values of 0.028 – 0.074 (average 0.041 ± 0.022) obtained in our study.

The presence of 3-F-RIF and RIF-Q may indicate the decomposition of RIF in biological samples during storage and preparation. In the present study, RIF-Q was determined in all patients' plasma and was in the range of 0.114 – 0.325 μg/mL (average 0.192 ± 0.082 μg/mL), comprising 2.30 – 4.50 % of the sum of RIF and RIF-Q. The peak of 3-F-RIF was detectable in all samples (Fig. 1), but the concentration of the compound was <LLOQ. Considering the possible interconversion of RIF and RIF-Q and pH changes during plasma storage, the degradation pathway of RIF requires further investigation.

Since there is a high demand for routine monitoring of anti-TB drugs [16], the practical guidelines on handling plasma or serum samples are

crucial for reliable results. Data on anti-TB drug stability in plasma or serum samples are extensive but equivocal in the context of TDM (Table 6). There are scarce data on the stability of 25-D-RIF [16,26,28]. To our best knowledge, this study is the first one presenting stability data for 3-F-RIF, and RIF-Q.

INH, PZA, and RIF are pretty stable in stock solutions; but in real samples, matrix components significantly impact INH and RIF stability. PZA was found to be stable on a benchtop for at least 4 h in plasma or serum samples [10,16,24,27,28], what was confirmed in our study. Some authors reported PZA stability in plasma or serum up to 24 h [25] at RT. On the contrary, INH was found unstable on the benchtop at RT in plasma or serum. Sturkenboom et al. observed that INH is significantly more stable in ultrafiltrate than in plasma and concluded that plasma proteins were involved in INH degradation [25]. While some authors reported INH stability during sample storage at RT for 12 h [18,19], the others observed a significant decay at RT in as short as 4 h [27,28]. Our results are in line with Kivrane et al. [28] and show 1 h stability of INH in plasma at RT. Data on RIF stability in plasma or serum samples are inconsistent. Some reports showed RIF samples stability at RT for as long as 24 h [8,34], while most authors reported lower RIF stability. [17] Le Guellec et al. [11] suggested better stability when RIF is stored in more concentrated solutions and when vitamin C is added as a preservative [11]. Our study proved that RIF and 3-F-RIF were stable in plasma samples when stored at RT for 4 h. However, we did not confirm 4 h stability of 25-D-RIF, as reported by Sundell et al. [16]. Our results showed 1 h stability of 25-D-RIF, which aligns with Kivrane et al. [28]. The data inconsistency on the RIF stability may be partially explained by the fact that RIF was found to be light-sensitive [35]. Additional uncontrolled exposure to light might be the case in the analysis of benchtop stability; it can be easily avoided by using amber glass and tubes for sample handling.

Unsatisfactory benchtop stability is not the only issue to address in the analysis of INH and RIF. Conditions for long-term sample storage and shipping have to be carefully considered. INH and RIF are stable in plasma or serum samples for 1 month [23,24,30] when stored at − 80 °C. There are conflicting data on longer storage at − 80 °C; Sundell et al. [16] reported that INH and RIF are stable for 12 weeks, while K im et al. [30] observed significant decay of both compounds during 12-week storage and confirmed only 4-week stability. Peloquin [12] reported RIF samples stability when stored for as long as 70 weeks at − 85 °C. However, in Peloquin's procedure, plasma was significantly processed before spiking the drug - it was charcoal-stripped, ultracentrifuged, and sequentially filtered [12]. Such handling would probably remove many matrix components leading to improved RIF stability.

Higher temperature for long-term storage seems risky, according to inconsistent stability data. Although plasma or serum samples containing PZA could be stored at − 20 °C for up to 3 months [28], INH and RIF can decay significantly in days. Kivrane et al. [28] observed degradation of INH when stored for 7 days. Our results show that INH is stable enough for 7-day storage at − 20 °C. Data on RIF stability at − 20 °C is very inconsistent; they range from < 1 week [11] to 3 months [28]. Our results support the findings of Le Guellec et al. [11] on the very poor stability of RIF as well as RIF-Q.

RIF, INH, and PZA were found stable during 3 cycles of freezing (at −70 °C or −80 °C) and thawing (at RT) [16,17,23,24]. Although Gao et al. [18] reported stability of INH, PZA, and RIF during 3 cycles of freezing at − 20 °C and thawing at RT; in similar conditions, Kivrane et al. [28] observed decay of INH and RIF, which we also observed in our study.

5. Conclusions

First-line anti-TB drugs such as INH, PZA, and RIF have been used in clinical practice for decades, and substantial data support TDM. However, the inconsistent data on sample stability could be confusing. The developed and validated UPLC-MS/MS method allowed detailed analysis of RIF stability and/or metabolism by quantifying 25-D-RIF, 3-F-RIF, and RIF-Q. Additionally, the usefulness of the method for TDM of INH, PZA, and RIF was confirmed in the analysis of clinical samples. The advantage of the method is a very low volume of plasma and fast and straightforward sample preparation.

According to the literature and the results of this study, we propose

Table 6
Literature data on stability of INH, PZA, RIF, and 25-D-RIF. Autosampler stability was not addressed since it is highly dependent on sample processing.

	RIF	INH	PZA	25-D-RIF
Stock solutions stability				
MeOH	1 mo at − 80 °C [23,24] 3 mo at − 20 °C [22] 3 mo at 4 °C [11]	1 mo at − 80 °C [23,24]	1 mo at − 80 °C [24]	—
MeOH:water (1:1, v/v)	—	—	1 mo at − 80 °C [23]	—
MeOH:water (5:95, v/v)	—	3 mo at − 20 °C [22]	3 mo at − 20 °C [22]	—
Working solutions stability				
MeOH	24 h at RT [24] 3 d at 4 °C [24] 3 mo at − 20 °C [22]	24 h at RT [24] 3 d at 4 °C [24] 3 mo at − 20 °C [22]	24 h at RT [24] 3 d at 4 °C [24] 3 mo at − 20 °C [22]	—
Plasma or serum samples				
Freeze-thaw stability	3 cycles [10,16–18,23,24,26,34]	3 cycles [10,16–19,23–26]	3 cycles [10,16–19,23–25,28]	3 cycles [16,26]
Benchtop / Short-term stability (RT)	2 h [11,17] 4 h [10,16,24,27,28] 5.5 h [12] 6 h [30] 12 h [18] 24 h [8,34]	1 h [28] 2 h [17,27] 4 h [10,16,24] 6 h [30] 12 h [18,19]	4 h [10,16,24,27,28] 6 h [30] 12 h [18,19] 18 h [17] 24 h [25]	1 h [28] 4 h [16]
Long-term stability at − 20 °C	< 1 wk [11] 2 wk [8] 20 d [20] 3 mo [28]	1 d [28] 20 d [20] 2 mo [19]	20 d [20] 51 d [25] 2 mo [19] 3 mo [28]	7 d [28]
at − 40 °C	30 d at − 40 °C [10]	30 d at − 40 °C [10]	30 d at − 40 °C [10]	—
at − 70 or − 80 °C	2 wk at − 70 °C [17] 18 d at − 70 °C [27] 1 mo at − 80 °C [23,24,30] 12 wk at − 80 °C [16] 70 wk at − 85 °C [12]	2 wk at − 70 °C [17] 18 d at − 70 °C [27] 1 mo at − 80 °C [23,24,30] 51 d at − 80 °C [25] 12 wk at − 80 °C [16]	2 wk at − 70 °C [17] 18 d at − 70 °C [27] 1 mo at − 80 °C [23,24] 51 d at − 80 °C [25] 12 wk at − 80 °C [16,30]	12 wk at − 80 °C [16]

Abbreviations: rifampicin (RIF), 25-desacetylrifampicin (25-D-RIF), isoniazid (INH), and pyrazinamide (PZA), MeOH – methanol, RT – room temperature

the following recommendations on plasma and serum sample handling for TDM of first-line antituberculars:

- Centrifuge the blood sample and collect plasma or serum as soon as possible; if short storage (minutes, not hours!) is needed – store at ~4 °C;
- The temperature-controlled centrifuge is preferred – centrifuge at low temperatures (preferably at ~4 °C);
- Process the plasma or serum sample quickly – it should not stand on the bench at room temperature for more than 1 h;
- Use amber glass and non-transparent tubes to protect samples from light;
- Store samples at − 80 °C for no longer than 1 month;
- Ship the samples frozen, with dry ice;
- Do not thaw the frozen sample more than three times.

CRediT authorship contribution statement

Marta Karaźniewicz-Łada: Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Validation; Visualization; Writing -Original draft preparation **Katarzyna Kosicka-Noworzyń**: Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Validation; Visualization; Writing -Original draft preparation **Prakruti Rao**: Data curation; Investigation **Nisha Modi**: Data curation; Investigation **Yingda L. Xie**: Data curation; Investigation; Funding acquisition; Writing Reviewing and Editing **Scott K. Heysell**: Data curation; Investigation; Resources; Funding acquisition; Writing Reviewing and Editing **Leonid Kagan**: Conceptualization; Funding acquisition; Project administration; Resources; Supervision; Writing -Original draft preparation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jpba.2023.115650.

References

[1] A. Alsultan, C.A. Peloquin, Therapeutic drug monitoring in the treatment of tuberculosis: an update, Drugs 74 (2014) 839–854, https://doi.org/10.1007/s40265-014-0222-8.
[2] C.A. Peloquin, Therapeutic drug monitoring in the treatment of tuberculosis, Drugs 62 (2002) 2169–2183, https://doi.org/10.2165/00003495-200262150-00001.
[3] M.A. Zuur, M.S. Bolhuis, R. Anthony, A. den Hertog, T. van der Laan, B. Wilffert, W. de Lange, D. van Soolingen, J.-W.C. Alffenaar, Current status and opportunities for therapeutic drug monitoring in the treatment of tuberculosis, Expert Opin. Drug Metab. Toxicol. 12 (2016) 509–521, https://doi.org/10.1517/17425255.2016.1162785.
[4] R. Choi, B.-H. Jeong, W.-J. Koh, S.-Y. Lee, Recommendations for optimizing tuberculosis treatment: therapeutic drug monitoring, pharmacogenetics, and nutritional status considerations, Ann. Lab. Med. 37 (2017) 97–107, https://doi.org/10.3343/alm.2017.37.2.97.
[5] A.-G. Märtson, G. Burch, S. Ghimire, J.-W.C. Alffenaar, C.A. Peloquin, Therapeutic drug monitoring in patients with tuberculosis and concurrent medical problems, Expert Opin. Drug Metab. Toxicol. 17 (2021) 23–39, https://doi.org/10.1080/17425255.2021.1836158.
[6] T. Sileshi, E. Tadesse, E. Makonnen, E. Aklillu, The impact of first-line anti-tubercular drugs' pharmacokinetics on treatment outcome: a systematic review, Clin. Pharmacol. 13 (2021) 1–12, https://doi.org/10.2147/CPAA.S289714.
[7] L. Mota, K. Al-Efraij, J.R. Campbell, V.J. Cook, F. Marra, J. Johnston, Therapeutic drug monitoring in anti-tuberculosis treatment: a systematic review and meta-analysis, Int. J. Tuberc. Lung Dis. 20 (2016) 819–826, https://doi.org/10.5588/ijtld.15.0803.
[8] P. Mishra, R.-P. Pawar, D. Bose, A. Durgbanshi, J. Albiol-Chiva, J. Peris-Vicente, J. Esteve-Romero, A. Jain, Stability studies of rifampicin in plasma and urine of tuberculosis patients according to the European medicines agency guidelines, Bioanalysis 11 (2019) 713–726, https://doi.org/10.4155/bio-2018-0174.
[9] I. Sutradhar, M.H. Zaman, Evaluation of the effect of temperature on the stability and antimicrobial activity of rifampicin quinone, J. Pharm. Biomed. Anal. 197 (2021), 113941, https://doi.org/10.1016/j.jpba.2021.113941.
[10] Y. Xing, L. Yin, X. Le, J. Chen, L. Zhang, Y. Li, H. Lu, L. Zhang, Simultaneous determination of first-line anti-tuberculosis drugs and one metabolite of isoniazid by liquid chromatography/tandem mass spectrometry in patients with human immunodeficiency virus-tuberculosis coinfection, Heliyon 7 (2021), e07532, https://doi.org/10.1016/j.heliyon.2021.e07532.
[11] C. Le Guellec, M.-L. Gaudet, S. Lamanetre, M. Breteau, Stability of rifampin in plasma: consequences for therapeutic monitoring and pharmacokinetic studies, Ther. Drug Monit. 19 (1997) 669–674.
[12] C.A. Peloquin, Rifampin stability, Ther. Drug Monit. 20 (1998) 450–451.
[13] C.J. Shishoo, S.A. Shah, I.S. Rathod, S.S. Savale, J.S. Kotecha, P.B. Shah, Stability of rifampicin in dissolution medium in presence of isoniazid, Int. J. Pharm. 190 (1999) 109–123, https://doi.org/10.1016/S0378-5173(99)00286-0.
[14] S. Singh, T.T. Mariappan, R. Shankar, N. Sarda, B. Singh, A critical review of the probable reasons for the poor variable bioavailability of rifampicin from anti-tubercular fixed-dose combination (FDC) products, and the likely solutions to the problem, Int. J. Pharm. 228 (2001) 5–17, https://doi.org/10.1016/S0378-5173(01)00754-2.
[15] J. Kuhlin, M.G.G. Sturkenboom, S. Ghimire, I. Margineanu, S.H.J. van den Elsen, N. Simbar, O.W. Akkerman, E.M. Jongedijk, R.A. Koster, J. Bruchfeld, D.J. Touw, J.-W.C. Alffenaar, Mass spectrometry for therapeutic drug monitoring of anti-tuberculosis drugs, Clin. Mass Spectrom. 14 (2019) 34–45, https://doi.org/10.1016/j.clinms.2018.10.002.
[16] J. Sundell, E. Bienvenu, S. Birgersson, A. Äbelö, M. Ashton, K.-J. Hoffmann, Simultaneous quantification of four first line antitubercular drugs and metabolites in human plasma by hydrophilic interaction chromatography and tandem mass spectrometry, J. Chromatogr. B 1105 (2019) 129–135, https://doi.org/10.1016/j.jchromb.2018.10.027.
[17] L. Wu, Z. Ye, H. Liu, H. Guo, J. Lin, L. Zheng, N. Chu, X. Liu, Rapid and highly sensitive quantification of the anti-tuberculosis agents isoniazid, ethambutol, pyrazinamide, rifampicin and rifabutin in human plasma by UPLC-MS/MS, J. Pharm. Biomed. Anal. 180 (2020), 113076, https://doi.org/10.1016/j.jpba.2019.113076.
[18] S. Gao, Z. Wang, X. Xie, C. You, Y. Yang, Y. Xi, W. Chen, Rapid and sensitive method for simultaneous determination of first-line anti-tuberculosis drugs in human plasma by HPLC-MS/MS: application to therapeutic drug monitoring, Tuberculosis 109 (2018) 28–34, https://doi.org/10.1016/j.tube.2017.11.012.
[19] K. Pršo, N. Žideková, I. Porvazník, I. Solovič, J. Mokrý, M. Kertys, A high-throughput LC–MS/MS method for simultaneous determination of isoniazid, ethambutol and pyrazinamide in human plasma, Rapid Commun. Mass Spectrom. 37 (2023), e9425, https://doi.org/10.1002/rcm.9425.
[20] X. Wang, H. Zhang, Y. Han, L. Huo, Y. Cao, X. Xu, L. Ai, Rapid and simultaneous determination of ten anti-tuberculosis drugs in human plasma by UPLC-MS/MS with applications in therapeutic drug monitoring, J. Chromatogr. B 1152 (2020), 122246, https://doi.org/10.1016/j.jchromb.2020.122246.
[21] J.B. Prahl, M. Lundqvist, J.M.C. Bahl, I.S. Johansen, Å.B. Andersen, N. Frimodt-Møller, A.S. Cohen, Simultaneous quantification of isoniazid, rifampicin, ethambutol and pyrazinamide by liquid chromatography/tandem mass spectrometry, APMIS 124 (2016) 1004–1015, https://doi.org/10.1111/apm.12590.
[22] S. Notari, C. Mancone, M. Sergi, F. Gullotta, N. Bevilacqua, M. Tempestilli, R. Urso, F.N. Lauria, L.P. Pucillo, M. Tripodi, P. Ascenzi, Determination of antituberculosis drug concentration in human plasma by MALDI-TOF/TOF, IUBMB Life 62 (2010) 387–393, https://doi.org/10.1002/iub.321.
[23] L. Baietto, A. Calcagno, I. Motta, K. Baruffi, V. Poretti, G. Di Perri, S. Bonora, A. D'Avolio, A UPLC-MS-MS method for the simultaneous quantification of first-line antituberculars in plasma and in PBMCs, J. Antimicrob. Chemother. 70 (2015) 2572–2575, https://doi.org/10.1093/jac/dkv148.
[24] Z. Zhou, X. Wu, Q. Wei, Y. Liu, P. Liu, A. Ma, F. Zou, Development and validation of a hydrophilic interaction liquid chromatography–tandem mass spectrometry method for the simultaneous determination of five first-line antituberculosis drugs in plasma, Anal. Bioanal. Chem. 405 (2013) 6323–6335, https://doi.org/10.1007/s00216-013-7049-0.
[25] M.G.G. Sturkenboom, H. van der Lijke, E.M. Jongedijk, B. W.Th. Kok, D.R. A. Greijdanus, J.-W.C. Uges, Alffenaar, Quantification of isoniazid, pyrazinamide and ethambutol in serum using liquid chromatography-tandem mass spectrometry, J. Appl. Bioanal. 1 (2015) 89–98, https://doi.org/10.17145/jab.15.015.
[26] K.H. Hee, J.J. Seo, L.S. Lee, Development and validation of liquid chromatography tandem mass spectrometry method for simultaneous quantification of first line

tuberculosis drugs and metabolites in human plasma and its application in clinical study, J. Pharm. Biomed. Anal. 102 (2015) 253–260, https://doi.org/10.1016/j.jpba.2014.09.019.

[27] L.T. Luyen, T.M. Hung, L.T. Huyen, L.A. Tuan, D.T.L. Huong, H.V. Duc, B.T. Tung, Simultaneous determination of pyrazinamide, rifampicin, ethambutol, isoniazid and Acetyl Isoniazid in human plasma by LC-MS/MS method, J. Appl. Pharm. Sci. 8 (2018) 61–73, https://doi.org/10.7324/JAPS.2018.8910.

[28] A. Kivrane, S. Grinberga, E. Sevostjanovs, V. Igumnova, I. Pole, A. Viksna, D. Bandere, A. Krams, A. Cirule, O. Pugovics, R. Ranka, LC-MS/MS method for simultaneous quantification of the first-line anti-tuberculosis drugs and six primary metabolites in patient plasma: implications for therapeutic drug monitoring, J. Chromatogr. B 1185 (2021), 122986, https://doi.org/10.1016/j.jchromb.2021.122986.

[29] S.H. Song, S.H. Jun, K.U. Park, Y. Yoon, J.H. Lee, J.Q. Kim, J. Song, Simultaneous determination of first-line anti-tuberculosis drugs and their major metabolic ratios by liquid chromatography/tandem mass spectrometry, Rapid Commun. Mass Spectrom. 21 (2007) 1331–1338, https://doi.org/10.1002/rcm.2961.

[30] H.-J. Kim, K.-A. Seo, H.-M. Kim, E.-S. Jeong, J.L. Ghim, S.H. Lee, Y.M. Lee, D. H. Kim, J.-G. Shin, Simple and accurate quantitative analysis of 20 anti-tuberculosis drugs in human plasma using liquid chromatography–electrospray ionization–tandem mass spectrometry, J. Pharm. Biomed. Anal. 102 (2015) 9–16, https://doi.org/10.1016/j.jpba.2014.08.026.

[31] S. Goutal, S. Auvity, T. Legrand, F. Hauquier, S. Cisternino, H. Chapy, W. Saba, N. Tournier, Validation of a simple HPLC-UV method for rifampicin determination in plasma: application to the study of rifampicin arteriovenous concentration gradient, J. Pharm. Biomed. Anal. 123 (2016) 173–178, https://doi.org/10.1016/j.jpba.2016.02.013.

[32] A.-G. Märtson, G. Burch, S. Ghimire, J.-W.C. Alffenaar, C.A. Peloquin, Therapeutic drug monitoring in patients with tuberculosis and concurrent medical problems, Expert Opin. Drug Metab. Toxicol. 17 (2021) 23–39, https://doi.org/10.1080/17425255.2021.1836158.

[33] T.J.F. Vanbrabant, A.C. Dijkmans, J. den Hartigh, D.J. Touw, S.M. Arend, Rifampin levels in daily practice: the accuracy of a single measurement, Neth. J. Med. 76 (2018) 235–242.

[34] F. de Velde, J.-W.C. Alffenaar, A.M.A. Wessels, B. Greijdanus, D.R.A. Uges, Simultaneous determination of clarithromycin, rifampicin and their main metabolites in human plasma by liquid chromatography–tandem mass spectrometry, J. Chromatogr. B 877 (2009) 1771–1777, https://doi.org/10.1016/j.jchromb.2009.04.038.

[35] R.C. Hartkoorn, S. Khoo, D.J. Back, J.F. Tjia, C.J. Waitt, M. Chaponda, G. Davies, A. Ardrey, S. Ashleigh, S.A. Ward, A rapid and sensitive HPLC–MS method for the detection of plasma and cellular rifampicin, J. Chromatogr. B 857 (2007) 76–82, https://doi.org/10.1016/j.jchromb.2007.07.005.