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Research article

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

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ABSTRACT

The incidence rate of tuberculosis (TB) in patients with human immunodeficiency virus (HIV) infection is 26 times higher than that in other patients. Patients with both infections require long-term combination therapy, which increases therapy complexity and might lead to serious adverse reactions and drug-drug interactions. To optimize therapy for patients with HIV and TB coinfection, we developed an ultra-high-performance liquid chromatography/tandem mass spectrometry (UHPLC-MS/MS) method to simultaneously quantify four anti-tuberculosis drugs and one isoniazid (INH) metabolite. Blood samples (n = 32) from 16 patients with HIV and TB coinfection were collected. Plasma protein precipitation with acetonitrile was followed by a hydrazine reaction between INH and cinnamaldehyde (CA) to produce phenylhydrazone (CA-INH) and dilution with heptafluorobutyric acid. The separation was performed on an Acquity UHPLC HSS T3 1.8 μm column (2.1 × 100 mm, Waters) with a mobile phase consisting of 10 mmol/L ammonium formate (pH = 4) in water (solvent A) and 0.1 % formic acid in methanol (solvent B) in a gradient elution. The compounds were detected using a positive multiple reaction monitoring model. INH, acetyl-INH (AC-INH), rifampicin (RIF), ethambutol (EMB), and pyrazinamide (PZA) showed good linear relationships in their quantitative ranges, with lower limits of quantification of 48, 192, 200, 96, and 480 ng/mL, respectively. The inter- and intraday precision was within 15 %, and the accuracy was between 85 % and 115 %. The mean plasma concentrations of INH, AC-INH, RIF, EMB, and PZA in patients were 1990.23 (24–16 600), 863.06 (96–2880), 3507.05 (229–9800), 808.10 (149–2130), and 18 838.33 (240–34 800) ng/mL, respectively. The plasma concentrations detected in the 16 patients were lower than the targeted concentrations in HIV-negative TB patients. In summary, we developed a simple UHPLC-MS/MS method for simultaneous quantification of first-line TB drugs, and successfully applied it for therapeutic drug monitoring in patients with HIV and TB coinfection. This method will facilitate monitoring of TB drugs in the future.

1. Introduction

With the widespread application of antiretroviral therapy, HIV is no longer the most deadly infectious disease. However, a high rate of virological failure (10–20 %) is still observed in patients receiving this therapy [1, 2, 3]. In addition, patients with acquired immune deficiency syndrome (AIDS) often suffer from a variety of opportunistic infectious diseases, of which tuberculosis (TB) is the most common. The incidence rate of TB in patients with AIDS is 26 times higher than that in other patients [4]. In patients with TB, HIV coinfection is present in 3.8 %–72.3 % [5, 6] of cases. Patients with both infections require long-term combination therapy, which can lead to poor compliance, multidrug resistance, and serious adverse reactions, such as liver dysfunction and thrombocytopenia [7, 8], and even the need to change the antiretroviral

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treatment protocol [9, 10]. Furthermore, there are potential drug-drug interactions in the treatment of AIDS complicated with TB [11, 12]. Moreover, the area under the curve (AUC) for first-line drugs for treating patients with HIV-TB coinfection is lower than that in patients with TB alone [13]. Therefore, individualized treatment is very important for patients with HIV-TB coinfection.

At present, the dominant first-line anti-TB drugs are isoniazid (INH), rifampicin (RIF), ethambutol (EMB), and pyrazinamide (PZA) [14]. For INH, the major active metabolite is acetyl-INH (AC-INH), which can also be monitored for INH dose adjustment [15]. Many studies have addressed plasma concentration monitoring for anti-TB drugs [8, 16, 17, 18]. Furthermore, because multidrug combined therapy is necessary for TB, a method to simultaneously monitor several anti-TB drugs in human plasma is needed. Several liquid chromatography (LC) or LC-tandem mass spectrometry (LC-MS/MS) methods for the measurement of first-line anti-TB drug levels in plasma have been reported [13, 19, 20, 21, 22, 23, 24, 25]. However, these methods have disadvantages, such as long chromatographic separation time [25], poor retention of INH, and partial separation of AC-INH and INH on reversed-phase columns [23].

In this study, we aimed to develop an ultra-high-performance liquid chromatography/tandem mass spectrometry (UHPLC-MS/MS) method to retain and separate four first-line anti-TB drugs and AC-INH on a reversed-phase column for the monitoring of plasma drug concentrations in patients with HIV-TB coinfection.

2. Materials and methods

2.1. Chemicals and reagents

Isoniazid-d4 (INH-d4), N-demethyl-rifampicin-d8 (N-demethyl-RIF-d8), ethambutol-d4 (EMB-d4), AC-INH, and acetyl isoniazid-d4 (AC–INH–d4) were purchased from Toronto Research Chemicals Inc. (Toronto, Canada). INH and EMB were obtained from the United States Pharmacopeia (Rockville, MD, USA), RIF from Dr. Ehrenstorfer (Augsburg, Germany), PZA from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), and pyrazinamide-d3 (PZA-d3) from TLC PharmaChem Inc. (Newmarket, Canada). HPLC-grade methanol and acetonitrile were purchased from ANPEL Laboratory Technologies (Shanghai, China), formic acid (FA) from Anaqua Chemicals Supply Company (Wilmington, Delaware, USA), and cinnamaldehyde (CA), vitamin C, and heptafluorobutyric acid (HFBA) from Sigma-Aldrich Company (Darmstadt, Germany). Ammonium formate (NH_4FA) was purchased from Honeywell (Charlotte, NC, USA). Blank plasma was collected from healthy volunteers, and the protocol was approved by Shanghai Public Health Clinical Center (2016-A020-01). Ultrapure water was produced in the laboratory using a Millipore system (Millipore, Bedford, MA, USA). INH-d4, N-demethyl-RIF-d8, EMB-d4, AC–INH–d4, and PZA-d3 were used as the internal standards (ISs) of INH, RIF, EMB, AC-INH, and PZA, respectively.

2.2. Volunteer enrollment and sample collection

The experimental protocol was approved by the Ethics Committee of the Shanghai Public Health Clinical Center (No. 2020-S177-02), and informed consent was obtained from all volunteers.

2.2.1. Healthy enrollment

Six healthy volunteers were enrolled, and 20 mL blood was collected from each individual into potassium ethylenediaminetetraacetic acid (EDTA-K_2)-anticoagulation tubes. The volunteers were selected according to the following criteria.

Inclusion criteria: age of 18–65 years, no HIV and TB infection, no TB drugs taken, and willingness to participate in the study.

Exclusion criteria: patients with cancer, pregnant, and breast-feeding.

2.2.2. Patient enrollment

This study was conducted at the Shanghai Public Health Clinical Center, Shanghai, China. Hospitalized adult HIV/TB -infected patients ($n = 16$) were enrolled from May to October 2020 according to the following criteria. Inclusion criteria: no less than 18 years old, HIV and TB co-infected, have taken TB drugs for at least seven days, and willing to participate in the study. Exclusion criteria: patients with cancer, pregnant, and breast-feeding.

Two blood samples (2 mL each) were collected at random time points from each patient in EDTA-K_2-anticoagulant tubes. After centrifugation, plasma was collected and frozen at −40 °C for further usage.

2.3. LC-MS/MS conditions

2.3.1. Optimizing mass spectrometry condition

The Waters Acquity UPLC system (Waters Corporation, Milford, MA, USA) coupled to an AB Sciex Triple Quad 5500 mass spectrometer (Applied Biosystems/AB SCIEX, Boston, MA, USA) was used for sample analysis. Quantification was achieved by multiple reaction monitoring in positive ion mode. The mass spectrometer was operated using the following settings: ion spray voltage, 5.5 kV; collision gas, medium; curtain gas, 40 psi; ion source gas 1 and ion source gas 2, 60 psi; entrance potential, 10 eV; collision cell exit potential, 13 eV. The declustering potential and collision energy (eV) were optimized as shown in Table 1.

2.3.2. Optimizing sample preparation and chromatography separation conditions

To improve hydrophobicity of INH, we derivatizated INH with cinnamaldehyde. To improve the retention of highly polar compounds

Table 1. Mass spectrometry parameters for the detection of analytes and their internal standards (ISs).

Compound (IS)[a]	Precursor ion (m/z)	Product ion (m/z)	Declustering potential (eV)	Collision energy (eV)
CA-INH	252.1	121.0	60	30
(CA–INH–d4)	256.1	125.1	70	20
AC-INH	180.1	138.2	90	20
(AC–INH–d4)	184.2	142.1	90	20
RIF	823.4	791.4	53	23
(N-demethyl-rifampicin-d8)	817.3	785.5	40	23
EMB	205.1	116.1	70	12
(EMB-d4)	209.1	120.2	57	12
PZA	124.0	81.0	60	25
(PZA-d3)	127.0	84.0	75	25

[a] IS, internal standard; CA-INH, isoniazid (INH) + cinnamaldehyde (CA); CA–INH–d4, INH-d4 + CA; AC-INH, acetyl isoniazid; AC–INH–d4, acetyl isoniazid-d4; RIF, rifampicin; N-demethyl-RIF-d8, N-demethyl-rifampicin-d8; EMB, ethambutol; EMB-d4, ethambutol-d4; PZA, pyrazinamide; PZA-d3, pyrazinamide-d3.

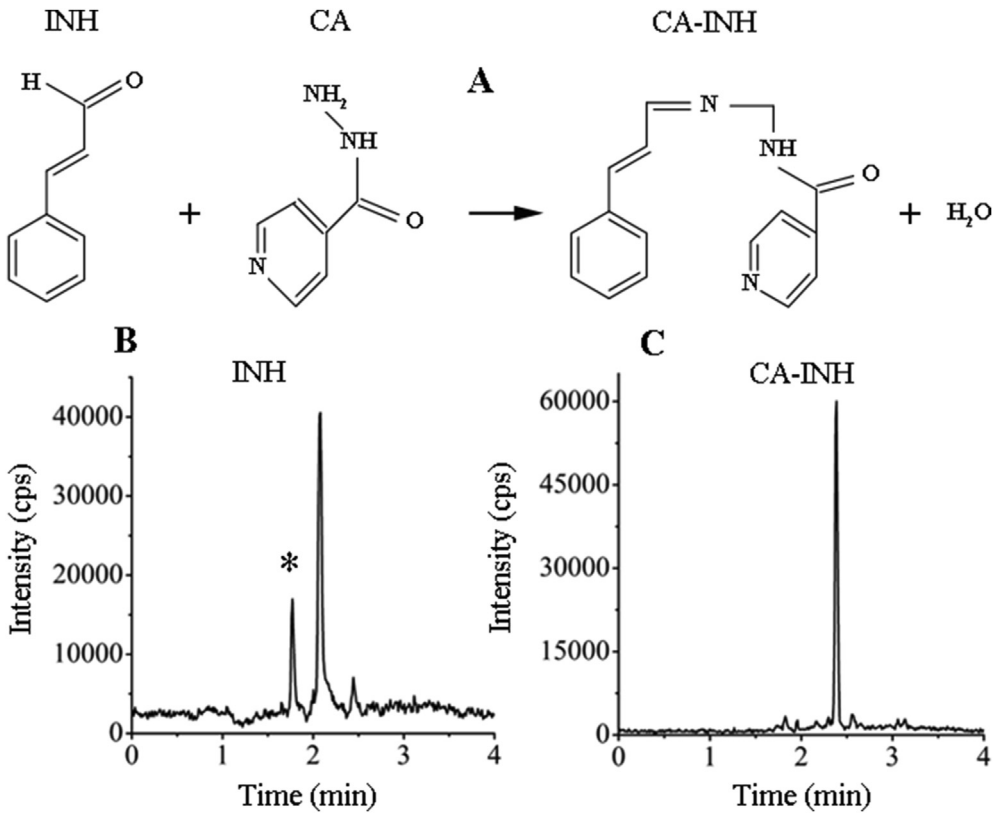

Figure 1. Reaction between isoniazid (INH) and cinnamaldehyde (CA) (A), and the chromatography profiles of INH (B) and CA-INH (C) CA-INH, the derivatization production of INH and CA. The peak was highlighted by asterisk (*).

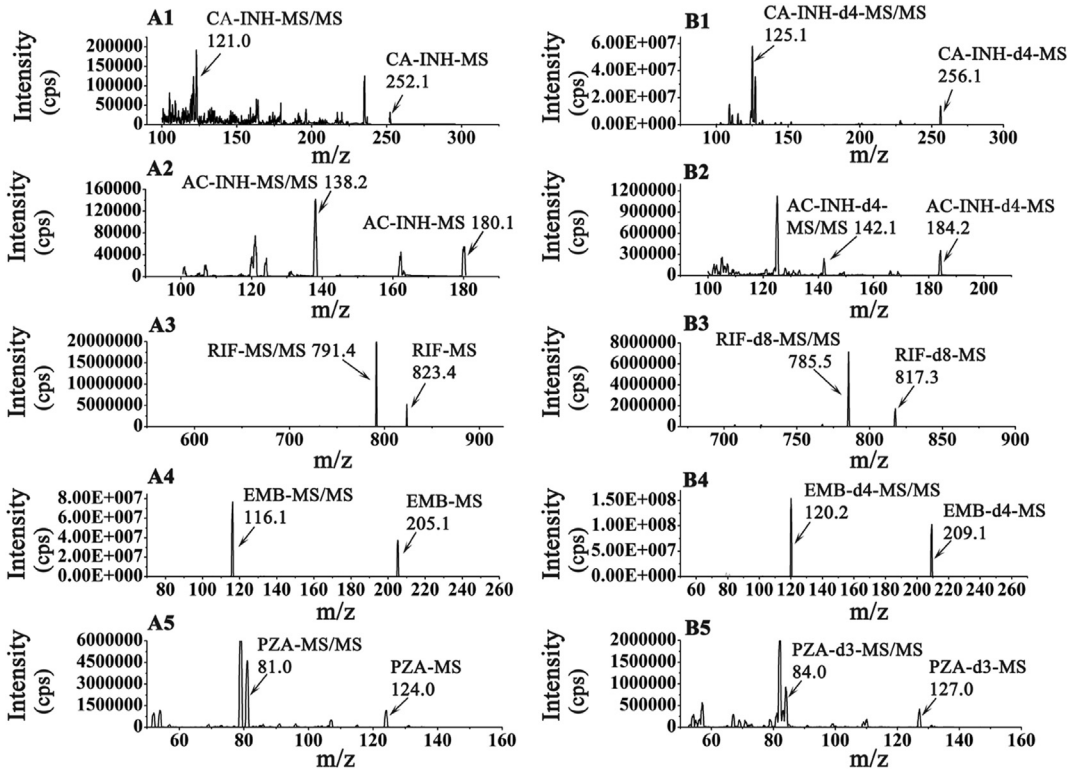

Figure 2. Electrospray product ion spectra (MS) of the five analytes (A) and their internal standards (B) A1, A2, A3, A4, and A5 show the peaks for the product ion spectra (MS) of CA-INH, AC-INH, RIF, EMB, and PZA, respectively. B1, B2, B3, B4, and B5 show those for the corresponding internal standards. AC-INH, acetyl isoniazid; CA-INH, isoniazid + cinnamaldehyde; INH, isoniazid; RIF, rifampicin; EMB, ethambutol; PZA, pyrazinamide.

in the chromatography column, we added ion-pairing reagent (HFBA) to the sample diluted solution. After optimizing the method for sample preparation, we compared the elution solution (B) of acetonitrile (ACN) and methanol, and solution A of 0.1% FA water, 5 mM NH_4FA, 5 mM NH_4FA (pH 4.0), 10 mM NH_4FA, and 10 mM NH_4FA (pH 4.0). Two kinds of spiked plasma standards with concentration of upper limit of quantification (ULOQ) and lower limit of quantification (LLOQ) were used. Chromatographic separation was performed on an Acquity UPLC HSS T3 1.8 μm column (2.1 × 100 mm, Waters) at a flow rate of 0.35 mL/min with HPLC gradient of 0–0.5 min 2% B, 1.5–2.5 min 80% B, and 3.0–4.0 min 2% B. The column temperature was 35 °C. For each sample, 5.0 μL was injected with a total running time of 4.0 min per sample.

2.4. Standard solutions and ISs

Analytes and their ISs were accurately weighed and dissolved separately in 90 % methanol (methanol:water 90:10, v/v) to obtain stock solutions with a concentration of 1 mg/mL. Then, the stock solutions for INH, RIF, EMB, PZA, and AC-INH were mixed and diluted with 50 % methanol to obtain a working solution (named S1) with the following concentrations: INH 60, AC-INH 240, RIF 250, EMB 120, and PZA 600 μg/mL. Then, S1 was diluted with 50 % methanol to obtain a series of working solutions. In total, seven concentrations of working standards were prepared, including INH at 60 000, 54 000, 24 000, 9600, 2400, 960, and 480 ng/mL; AC-INH at 240 000, 216 000, 96 000, 38 400, 9600, 3840, and 1920 ng/mL; RIF at 250 000, 225 000, 100 000, 40 000, 10

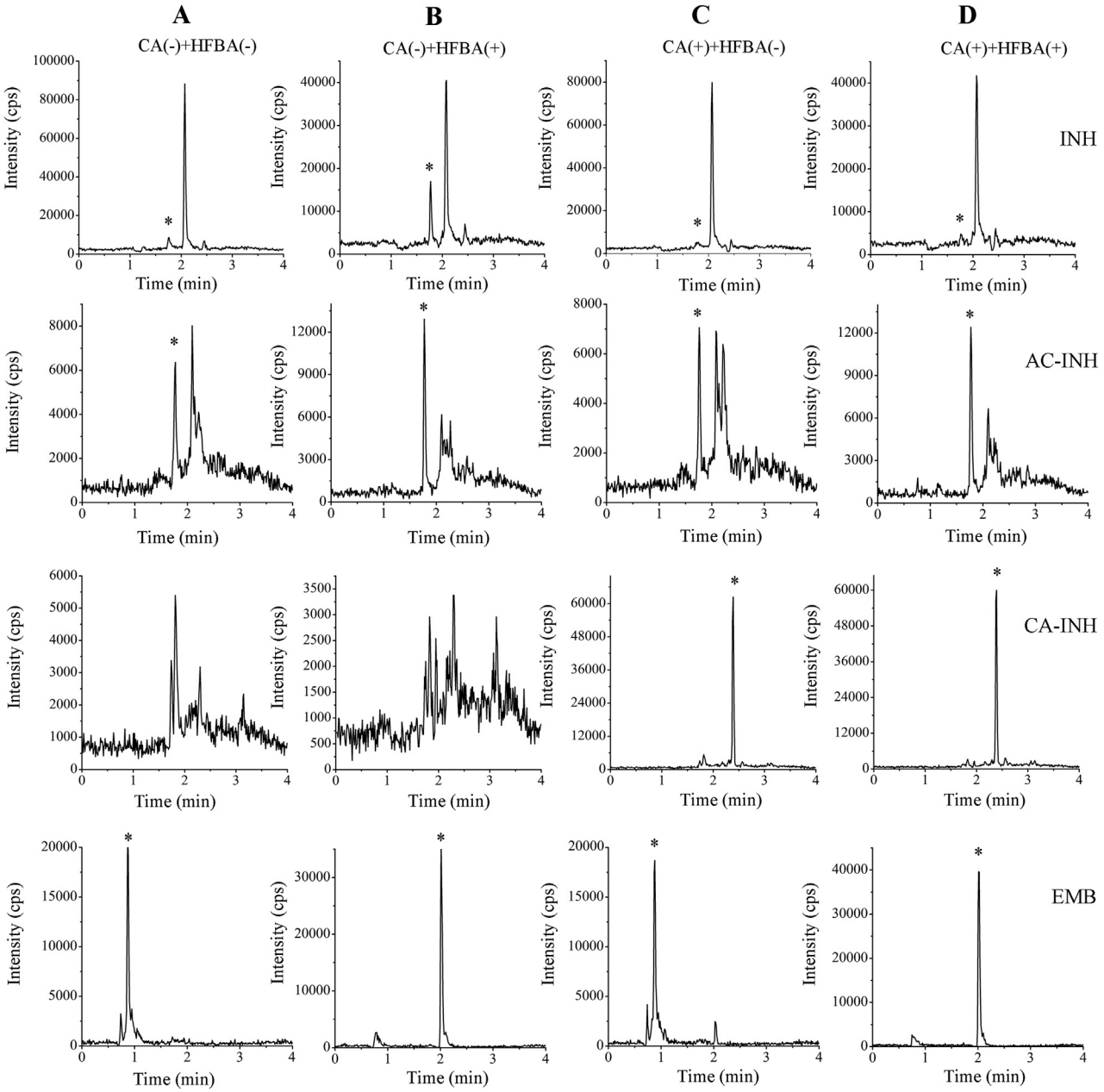

Figure 3. The affected chromatograms of CA-INH, AC-INH, and EMB with or without CA and HFBA addition. A. without CA and HFBA (marked as (CA(-)+HFBA(-))); B. without CA and with HFBA (marked as (CA(-)+HFBA(+))); C. with CA and without HFBA (marked as (CA(+)+HFBA(-))); D. with CA and HFBA (marked as (CA(+)+HFBA(+)). AC-INH, acetyl isoniazid; CA-INH, isoniazid + cinnamaldehyde; EMB, ethambutol; CA, cinnamaldehyde; HFBA, heptafluorobutyric acid. The peaks of each compound were highlighted by asterisks (*).

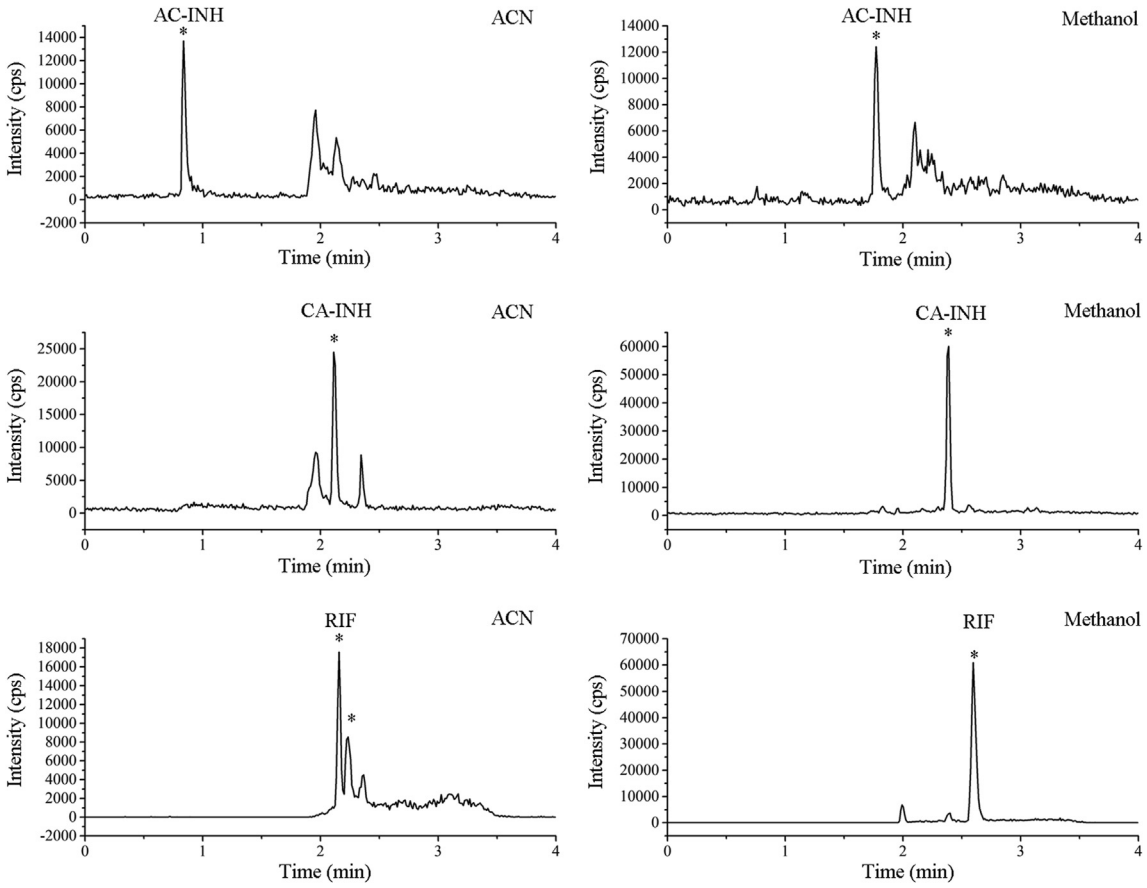

Figure 4. The chromatograms of AC-INH, CA-INH, and RIF eluted by differential organic phases in the spiked standard of lower limit of quantification (LLOQ). AC-INH, acetyl isoniazid; CA-INH, isoniazid + cinnamaldehyde; RIF, rifampicin. The peaks of each compound were highlighted by asterisks (*).

000, 4000, and 2000 ng/mL; EMB at 120 000, 108 000, 48 000, 19 200, 4800, 1920, and 960 ng/mL; and PZA at 600 000, 540 000, 240 000, 96 000, 24 000, 9600, and 4800 ng/mL. Four types of quality control (QC) working solutions were prepared in the same way at concentrations of 48 000, 19 200, 1200, and 480 μg/mL for INH; 200 000, 80 000, 5000, and 2000 μg/mL for RIF; 192 000, 76 800, 4800, and 1920 μg/mL for AC-INH; 96 000, 38 400, 2400, and 960 μg/mL for EMB; and 480 000, 192 000, 12 000, and 4800 μg/mL for PZA. The working standards were combined with blank plasma at 10-fold dilutions to obtain calibration standards.

Stock solutions of ISs were also combined and diluted with 50 % methanol to obtain the working IS solution with INH-d4 (5 μg/mL), AC–INH–d4 (10 μg/mL), N-demethyl-RIF-d8 (100 μg/mL), EMB-d4 (6 μg/mL), and PZA-d3 (250 μg/mL). All solutions were stored at −40 °C.

2.5. Sample preparation

Calibration standards or patient plasma (50 μL) were mixed with 20 μL of IS working solution, 20 μL of 0.5 % vitamin C, and 300 μL of acetonitrile. After vortexing for 3 min, the mixtures were centrifuged at 4 °C and 12 000 rpm for 10 min. The supernatant (40 μL) was mixed with 4 % CA (40 μL of each) to produce the hydrazone between INH and CA. After vortexing and allowing the sample to stand at room temperature (20–25 °C) for 30 min, 360 μL of diluent (20 % acetonitrile, 0.1 % FA, and 2 % HFBA) was added. After vortexing again, 5 μL of this mixture was injected into the UHPLC for analysis.

2.6. Method validation

The method validation was followed the US Food and Drug Administration guidelines.

2.6.1. Specificity

Three types of plasma samples (blank plasma, spiked calibration standard, and patient plasma) were prepared and analyzed as described in section 2.4. The specificity of the method was verified by comparing the chromatogram profiles of these three sample types.

2.6.2. Limits of detection and quantification (LOD and LOQ)

The LOD was estimated as the concentration level resulting in a peak area of three times the baseline noise measured in blank plasma extracts at the retention times of the analytes, and the LOQ was determined as the limit of quantification at which analytes/signals were 10. Six replicates were prepared.

2.6.3. Calibration curves

The linear ranges of the five compounds were INH 48–6000 ng/mL, AC-INH 192–24 000 ng/mL, RIF 200–25 000 ng/mL, EMB 96–12 000 ng/mL, and PZA 480–60 000 ng/mL. The lower limit of quantitation (LLOQ) was defined as the lowest concentration of the calibration standards with a precision <20 % and accuracy within ±20 %. The x-coordinate was the concentration of the analyte, and the y-coordinate was the area ratio of the analyte to the internal standard peak. The calibration curve was obtained by linear regression with weighted least squares (weighted factor $1/\rho^2$).

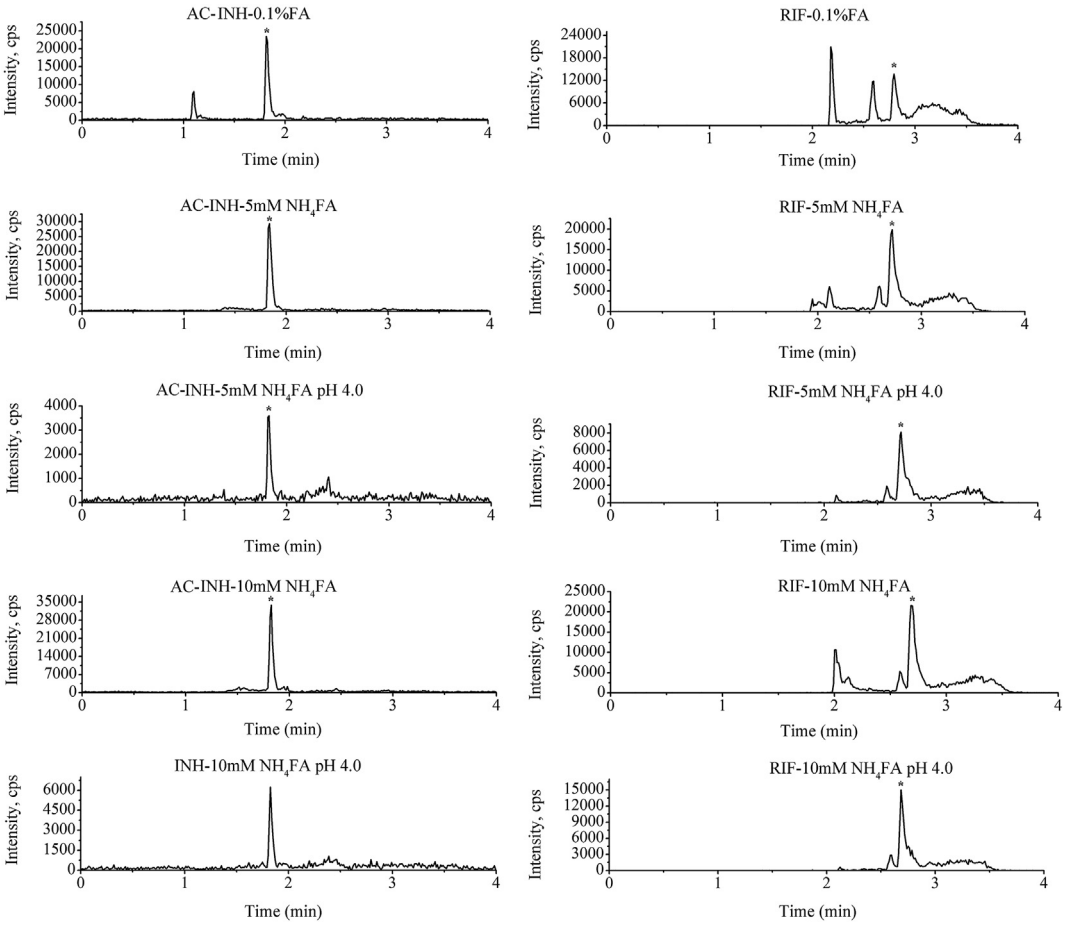

Figure 5. The chromatograms of INH, AC-INH, RIF, EMB, and PZA eluted by differential aqueous phases in the spiked standard of lower limit of quantification (LLOQ). The peaks of each compound were highlighted by asterisks (*).

2.6.4. Inter- and intraday accuracy and precision

Four types of QC samples were prepared with six replicates for each: high (HQC), medium (MQC), low (LQC), and the LLOQ. The samples were processed with the same sample preparation method, and three analytical batches were continuously assessed to obtain the inter- and intraday precision and accuracy.

2.6.5. Extraction recovery and matrix effect

The extraction recovery and matrix effect were evaluated at three QC levels (HQC, MQC, and LQC) with six replicates. The extraction recovery and matrix effect were obtained by calculating the peak area ratio for the spiked sample to the spiked extraction sample, and for the spiked extraction sample to the solvent-substituted sample at the same nominal concentration, respectively.

2.6.6. Stability

The stability of analytes in plasma was investigated with the HQC and LQC samples (6 replicates for each) at room temperature for 4 h, at 4 °C for 4 h, in the autosampler for 24 h after sample processing, for three freeze-thaw cycles from −40 °C to room temperature, and for long-term storage for 30 d at −40 °C.

2.7. Application of the UHPLC-MS/MS method

The validated UHPLC-MS/MS method was applied for the simultaneous determination of first-line anti-TB drugs (INH, RIF, EMB, and PZA) and AC-INH in patients with HIV-TB coinfection.

2.8. Statistical analysis

Data are presented as means and range or scatter plots showing individual values. Plasma analyte concentrations below the quantifiable limit were recorded as 1/2 LLOQ [26].

3. Results

3.1. LC-MS method development and validation

3.1.1. LC-MS method

To improve the retention of INH (soluble in water) in the column and better separate INH and AC-INH, INH was reacted with CA to produce the hydrazone (Figure 1A). INH and INH-d4 were detected to have a pair of ions at *m/z* of 252.1–121.0 (CA-INH, Figure 2 A1) and 256.1–125.1 (CA–INH–d4, Figure 2 B1), respectively. The other four analytes and their ISs were hydrogen adduct ions ($[M + H]^+$). Typical MS profiles are shown in Figure 2 A2–A5 for the four analytes, and Figure 2 B2–B5 for their ISs.

After derivation of INH with CA, the retention time of INH was increased with intensity improving significantly (Figure 3). Furthermore, with the addition of HFBA, AC-INH was eluted as a single peak, and the retention time of EMB was increased to 2.1 min from less than 1 min (Figure 3).

After optimizing the sample preparation conditions, we compared the mobile phase B (ACN or methanol containing 0.1% FA) (Figure 4) and found that AC-INH could retain poorly, and CA-INH and RIF had multiple peaks in the column using ACN containing 0.1% FA. Then we

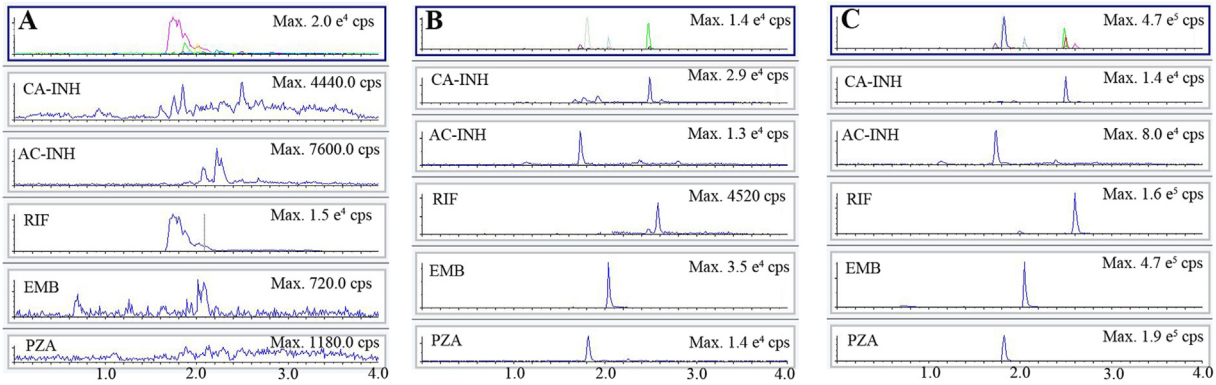

Figure 6. Representative multiple reaction monitoring chromatograms for CA-INH, AC-INH, RIF, EMB, and PZA in human plasma. (A) Blank plasma sample; (B) blank plasma sample spiked with five analytes at the LLOQ concentration; (C) plasma sample from patient with HIV-TB coinfection. AC-INH, acetyl isoniazid; CA-INH, isoniazid + cinnamaldehyde; INH, isoniazid; RIF, rifampicin; EMB, ethambutol; PZA, pyrazinamide.

Table 2. The limit of detection (LOD) by the UHPLC-MS/MS method.

Analytes	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	Average
AC-INH	4	3.8	3.1	3.9	4	2.1	3.5
PZA	3.5	7	8.3	5.9	9.3	3.8	6.3
EMB	57	45.5	35.4	57.8	27.3	38.3	43.6
CA-INH	5	10.5	10	10.2	9.5	14.1	9.9
RIF	5	3.5	3	4.6	4	3.5	3.9

No. 1 to 6 represent six replicates.

fixed B (methanol containing 0.1% FA), compared aqueous mobile A, and found that 10 mM NH_4FA (pH = 4.0) was the best for the elution of AC-INH and RIF (Figure 5) with no significant difference for the other compounds. The retention times of CA-INH, AC-INH, RIF, EMB, and PZA were approximately 2.5, 1.7, 2.6, 2.1, and 1.8 min, respectively (Figure 6). As shown in Figure 6, all analytes were retained and separated well. No interfering endogenous substances were detected in the blank plasma, which demonstrates that the specificity of this method is acceptable.

3.1.2. LC-MS method validation

The LOD of AC-INH, PZA, EMB, CA-INH, and RIF, were 1/3 of the LLOQ with signal to noise (S/N) ratios of about 3.5, 6.3, 43.6, 9.9, and 3.9, respectively (Table 2). The LOQ was equal to LLOQ.

Table 3. Inter- and intraday precision and accuracy for the analytes in human plasma.

Analytes	Concentration (ng/mL)[a]	Intra-day			Inter-day		
		Measured concentration (ng/mL)	Precision (%)	Accuracy (%)	Measured concentration (ng/mL)	Precision (%)	Accuracy (%)
INH	48	45.93	7.18	95.71	45.28	6.91	94.36
	120	118.83	3.17	98.98	121.50	4.01	101.28
	1920	2003.33	3.62	104.36	1956.67	4.88	101.89
	4800	5003.33	3.18	104.22	4874.44	3.24	101.59
RIF	200	207.67	11.68	103.80	189.78	12.11	94.91
	500	532.50	6.71	106.49	524.06	7.65	104.83
	8000	8831.67	4.38	110.40	8210.56	7.27	102.64
	20000	21633.33	7.37	108.22	20200	7.14	100.99
EMB	96	86.68	8.43	90.30	84.85	6.81	88.38
	240	236.33	4.17	98.36	239.11	4.69	99.61
	3840	4023.33	3.24	104.77	3900	4.19	103.89
	9600	10030	4.70	104.58	9650	7.27	100.55
PZA	480	482.67	8.14	100.54	457.78	7.43	95.34
	1200	1238.33	2.67	103.22	1226.11	5.07	102.16
	19200	19566.67	3.29	101.92	19288.89	2.81	100.46
	48000	47816.67	1.80	99.61	47927.78	2.80	99.85
AC-INH	192	201.33	11.63	104.91	188.56	10.21	98.23
	480	476.17	5.32	99.20	480.50	5.70	100.13
	7680	8001.67	5.29	104.20	7829.44	4.42	101.94
	19200	19300	4.16	100.51	19188.89	2.97	99.96

[a] The QC samples from top to bottom are the LLOQ, LQC, MQC, and HQC.

Table 4. Extraction recovery and matrix effect for the analytes in human plasma.

Analytes	Concentration (ng/mL)[a]	Extraction recovery		Matrix effect	
		Mean (%)	RSD (%)	Mean (%)	RSD (%)
INH	120	94.18	3.78	94.91	2.86
	1920	97.20	2.17	92.39	3.23
	4800	95.94	3.00	88.23	4.06
AC-INH	480	84.24	3.40	95.53	4.18
	7680	97.41	2.64	99.66	3.05
	19 200	94.15	3.41	103.24	5.26
RIF	500	99.59	9.87	97.36	4.53
	8000	98.66	3.35	95.89	3.26
	20 000	99.91	3.11	98.05	2.16
EMB	240	93.83	2.44	98.02	3.08
	3840	93.83	1.19	99.86	3.71
	9600	93.36	4.53	94.92	2.58
PZA	1200	97.46	3.33	97.38	3.42
	19 200	98.60	2.49	98.61	3.58
	48 000	97.52	2.78	101.36	2.08

[a] The QC samples from top to bottom are the LQC, MQC, and HQC.

CA-INH, AC-INH, RIF, EMB, and PZA showed linear relationships in the ranges of 480–6000 ng/mL, 192–24 000 ng/mL, 200–25 000 ng/m, 96–12 000 ng/mL, and 48–60 000 ng/mL, respectively. The inter- and intraday precision for the HQC, MQC, LQC, and LLOQ were all less than 15.00 %, and the accuracy ranged from 85.00% to 115.00% (except for the LLOQ) (Table 3).

3.1.3. Extraction recovery and matrix effect

The ranges of the extraction recovery and matrix effect for all analytes were 84.24%–99.91% and 88.23%–103.24%, respectively (Table 4).

3.1.4. Stability

The stability of INH, AC-INH, RIF, EMB, and PZA in human plasma was investigated. The analytes were stable under different storage conditions with deviations within ±15% (Table 5).

3.2. Clinical information and drug concentrations in patient samples

The validated UHPLC-MS/MS method was successfully applied to detect the plasma drug concentrations of INH, AC-INH, RIF, EMB, and PZA in patients with HIV-TB coinfection. In this study, 16 patients were enrolled, including 15 men and 1 woman, with an average age of 46.69 (27–72) years. Two samples were taken at random time points from each patient; therefore, 32 samples were collected. The mean plasma concentrations of INH, AC-INH, RIF, EMB, and PZA were 1990.23 (24–16 600), 863.06 (96–2880), 3507.05 (229–9800), 808.10 (149–2130), and 18 838.33 (240–34 800) ng/mL, respectively. The detailed clinical information and drug concentrations are shown in Table 6 and Figure 7.

4. Discussion

The main reason for the poor treatment outcomes in patients with drug-susceptible TB may be the lower-than-expected plasma concentrations of first-line anti-TB drugs [17, 27], especially in patients with HIV-TB coinfection [17]. As noted by Daskapan, HIV affects the C_{max} and AUC of anti-TB drugs; for example, HIV reduces the AUC of RIF and C_{max} of INH [28, 29, 30]. In patients with TB, C_{max} values of daily doses in HIV-positive patients were 20% lower than those in HIV-negative patients [31]. More than 60% of patients have low C_{max} for RIF and EMB [32].

To improve the clinical effect, therapeutic drug monitoring (TDM) has been widely performed to optimize drug doses in patients infected with TB, especially with HIV-TB [33, 34, 35, 36]. To perform TDM, several methods have been developed to quantify drug concentration, including gas chromatography (GC) [37], GC-MS [31], high performance liquid chromatography [38, 39], and LC-MS/MS [14, 40]. However, most of them have disadvantages, such as long analysis time [14, 25, 38] (as long as 26 min) [38] and poor chromatograph retention [19, 23, 40, 41].

Table 5. Stability of analytes in human plasma.

Analytes	Concentration (ng/mL)[a]	Stability (RSD%)				
		Short-term (4 h at room temperature)	Short-term (4 h at 4 °C)	Long-term (30 d at -40 °C)	24 h at auto-sampler (4 °C)	Three freeze-thaw
INH	120	5.27	7.81	10.01	10.59	10.81
	4800	7.55	7.29	7.69	5.84	9.25
RIF	500	11.67	7.07	13.14	4.38	11.27
	20 000	8.74	5.14	8.75	6.84	8.06
EMB	240	3.54	4.73	5.84	3.04	4.90
	9600	6.77	6.95	6.53	6.72	6.34
PZA	1200	1.62	2.70	2.25	2.03	3.18
	48 000	2.00	3.46	3.24	2.28	2.33
AC-INH	480	5.79	8.17	4.75	2.71	5.88
	19 200	5.08	7.72	3.32	5.04	6.06

[a] The top and bottom QC samples are the LQC and HQC, respectively.

Table 6. Clinical information and drug concentrations in patients with TB.

No.	Age (y)	Sex	Weight	GFR (mg/mL)	Cr (μmol/L)	ALT (U/L)	AST (U/L)	Concentration (ng/mL)				
								INH*	AC-INH*	RIF	EMB	PZA*
1	36	F	40	157.579	36.38	7	12	24	ND	ND	187	6340
								319	96	ND	347	7570
2	28	M	45	198.124	46.29	11	14	ND	ND	7190	1740	ND
								ND	ND	6050	1890	ND
3	39	M	50	164.959	51.27	15	9	ND	ND	3660	429	ND
								ND	ND	1590	307	ND
4	40	M	50	125.781	64.85	37	30	35	96	463	540	30900
								24	ND	ND	311	22300
5	27	M	60	271.573	24.03	8	15	24	ND	1830	149	4400
								16600	2110	229	1170	28500
6	42	M	53.7	103.846	75.91	22	20	70.7	ND	ND	694	ND
								2120	768	953	607	ND
7	50	M	45	311.587	28.41	8	20	24	ND	9800	1230	ND
								ND	ND	4840	1100	ND
8	35	M	40	318.622	29.67	23	27	24	96	899	183	ND
								24	ND	634	183	ND
9	56	M	60	155.643	50.82	41	37	45.5	848	1320	794	31900
								24	491	713	595	28900
10	68	M	60	190.895	41.15	43	43	47.5	613	4790	364	29900
								12100	2880	3280	667	34800
11	51	M	52	219.322	38.38	13	20	24	ND	ND	2130	27500
								6590	1610	ND	1230	20000
12	72	M	62	91.702	76.9	21	22	406	248	9310	450	18700
								1830	1140	3710	1630	25300
13	35	M	60	155.401	51.05	18	21	308	96	4720	237	9100
								864	753	4160	1080	12500
14	36	M	48	127.198	65.11	40	38	24	ND	ND	ND	ND
								24	ND	ND	ND	ND
15	68	M	60	149.492	50.86	10	7	ND	ND	ND	587	ND
								ND	ND	ND	870	ND
16	64	M	58	163.294	50.09	38	26	4520	924	ND	942	240
								3660	1040	ND	1600	240
Average	46.7		52.7	181.56	48.823	22.19	22.56	1990.23	863.06	3507.05	808.10	18838.33
Min	27		40	91.702	24.03	7	7	24	96	229	149	240
Max	72		62	318.622	76.9	43	43	16600	2880	9800	2130	34800

ND: not detected. GFR, glomerular filtration rate; Cr, plasma creatinine; ALT, Alanine aminotransferase; AST, aspartate aminotransferase.

* For the concentration less than LLOQ, 1/2 LLOQ was recorded, with 24 ng/mL for INH, 96 ng/mL for AC-INH, and 240 ng/mL for PZA.

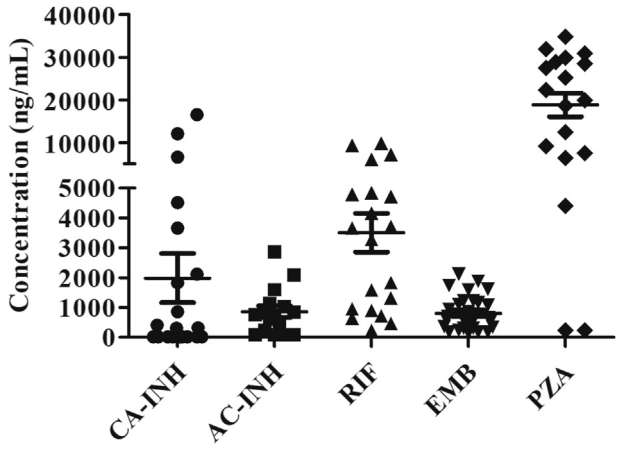

Figure 7. Measured plasma concentrations of INH, AC-INH, RIF, EMB, and PZA in patients with HIV-TB coinfection. AC-INH, acetyl isoniazid; INH, isoniazid; RIF, rifampicin; EMB, ethambutol; PZA, pyrazinamide.

For example, in a previous study, the retention and peak shape of EMB were not favorable [19]. INH and AC-INH have weak retention and cannot be separated very well [23] or have a longer retention time [25].

In this study, INH was derivatized to phenylhydrazone by CA, which significantly increased its hydrophobicity. The reaction between INH and CA is simple, and only requires incubation at room temperature for about 30 min. Furthermore, to improve the retention of strong polar compounds, HFBA was added to the dilution solution. In a previous study, HFBA (0.02%) was added to the mobile phase [40], which might affect the detection of negatively ionized compounds. Therefore, this effect will be less notable in our method than in the reported method [40]. Furthermore, RIF might be self-oxidized to rifampicin quinone [42]. In this work, vitamin C was used to prevent the oxidation of RIF into benzoquinone [43, 44].

The developed method was successfully used to detect plasma drug concentrations in patients with HIV-TB coinfection. The average concentrations detected in this study were much lower than the target concentrations for INH (3–6 μg/mL), RIF (8–24 μg/mL), PZA (20–60 μg/mL), and EMB (2–6 μg/mL) [34]. The results from our study are in good agreement with a previous report indicating that RIF and EMB have a significantly lower C_{max} in HIV/TB than in TB alone [13]. The C_{max} of

EMB in HIV-positive patients was 20% lower than that in HIV-negative patients [31]. Furthermore, some concentrations detected in this work were very low, which might be due to the random sampling. The trough concentrations were much lower than the peaks [41], with that of INH being less than 0.25 μg/mL [45] and that of RIF being 0.6 (0.1–10.3) μg/mL [46]. The lower drug concentrations might also be due to intra and inter-individual variability in patients. As shown in Table 5, 87.5% of the patients had a GFR higher than the normal range of 80–120 mL/min. All patients had creatinine clearance rates of less than 80–120 mL. The ALT and AST levels in all patients were normal (less than 50 U/L). In summary, variabilities in the concentrations of TB drugs might not only be due to the interaction between antiretrovirals and TB drugs, but also intra and inter-individual variability (such as in Cr and GFR). In the future, it is necessary to perform PK/PD study in large sample clinical trials.

5. Conclusion

In this study, we developed a UHPLC-MS/MS method to simultaneously quantify first-line anti-TB drugs and a metabolite of INH (AC-INH). This method was successfully applied to monitor plasma drug concentrations in patients with HIV and TB. The concentrations in patients with HIV-TB coinfection were lower than those in patients with TB who were HIV-negative according to a previous report [34]. One limitation of this study is that the samples were collected at random times. Further, well-designed TDM studies with a large cohort must be performed.

Declarations

Author contribution statement

Yaru Xing: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Lin Yin; Xiaoqin Le; Jun Chen; Lin Zhang: Performed the experiments; Analyzed and interpreted the data.

Yingying Li: Analyzed and interpreted the data.

Hongzhou Lu: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Lijun Zhang: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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References

[1] E.K. Hester, K. Astle, Dolutegravir-Rilpivirine, Dual antiretroviral therapy for the treatment of HIV-1 infection, Ann. Pharmacother. 53 (2019) 860–866.
[2] J. Reynes, R. Trinh, F. Pulido, R. Soto-Malave, J. Gathe, R. Qaqish, M. Tian, L. Fredrick, T. Podsadecki, M. Norton, A. Nilius, Lopinavir/ritonavir combined with raltegravir or tenofovir/emtricitabine in antiretroviral-naive subjects: 96-week results of the PROGRESS study, AIDS Res. Hum. Retrovir. 29 (2013) 256–265.
[3] N. Ngo-Giang-Huong, L. Wittkop, A. Judd, P. Reiss, T. Goetghebuer, D. Duiculescu, A. Noguera-Julian, M. Marczynska, C. Giacquinto, L. Ene, J.T. Ramos, C. Cellerai, T. Klimkait, B. Brichard, N. Valerius, C. Sabin, R. Teira, N. Obel, C. Stephan, S. de Wit, C. Thorne, D. Gibb, C. Schwimmer, M.A. Campbell, D. Pillay, M. Lallemant, EuroCoord, Prevalence and effect of pre-treatment drug resistance on the virological response to antiretroviral treatment initiated in HIV-infected children - a EuroCoord-CHAIN-EPPICC joint project, BMC Infect. Dis. 16 (2016) 654.
[4] J.A. Tornheim, K.E. Dooley, Challenges of TB and HIV co-treatment: updates and insights, Curr. Opin. HIV AIDS 13 (2018) 486–491.
[5] J. Gao, P. Zheng, H. Fu, Prevalence of TB/HIV co-infection in countries except China: a systematic review and meta-analysis, PloS One 8 (2013), e64915.
[6] A.W. Denegetu, B.L. Dolamo, HIV screening among TB patients and co-trimoxazole preventive therapy for TB/HIV patients in Addis Ababa: facility based descriptive study, PloS One 9 (2014), e86614.
[7] E.J. Forget, D. Menzies, Adverse reactions to first-line antituberculosis drugs, Expet Opin. Drug Saf. 5 (2006) 231–249.
[8] J. Reynolds, S.K. Heysell, Understanding pharmacokinetics to improve tuberculosis treatment outcome, Expet Opin. Drug Metabol. Toxicol. 10 (2014) 813–823.
[9] S. Kanters, M.E. Socias, N.I. Paton, M. Vitoria, M. Doherty, D. Ayers, E. Popoff, K. Chan, D.A. Cooper, M.O. Wiens, A. Calmy, N. Ford, S. Nsanzimana, E.J. Mills, Comparative efficacy and safety of second-line antiretroviral therapy for treatment of HIV/AIDS: a systematic review and network meta-analysis, Lancet HIV 4 (2017) e433–e441.
[10] J. Troya, J. Bascunana, Safety and tolerability: current challenges to antiretroviral therapy for the long-term management of HIV infection, AIDS Rev. 18 (2016) 127–137.
[11] M. Weiner, D. Benator, C.A. Peloquin, W. Burman, A. Vernon, M. Engle, A. Khan, Z. Zhao, C. Tuberculosis Trials, Evaluation of the drug interaction between rifabutin and efavirenz in patients with HIV infection and tuberculosis, Clin. Infect. Dis. 41 (2005) 1343–1349.
[12] D.A. Benator, M.H. Weiner, W.J. Burman, A.A. Vernon, Z.A. Zhao, A.E. Khan, B.E. Jones, L. Sandman, M. Engle, C. Silva-Trigo, P.H. Hsyu, M.I. Becker, C.A. Peloquin, C. Tuberculosis Trials, Clinical evaluation of the nelfinavir-rifabutin interaction in patients with tuberculosis and human immunodeficiency virus infection, Pharmacotherapy 27 (2007) 793–800.
[13] S. Antwi, H. Yang, A. Enimil, A.M. Sarfo, F.S. Gillani, D. Ansong, A. Dompreh, A. Orstin, T. Opoku, D. Bosomtwe, L. Wiesner, J. Norman, C.A. Peloquin, A. Kwara, Pharmacokinetics of the first-line antituberculosis drugs in Ghanaian children with tuberculosis with or without HIV coinfection, Antimicrob. Agents Chemother. 61 (2017).
[14] J.B. Prahl, M. Lundqvist, J.M. Bahl, I.S. Johansen, A.B. Andersen, N. Frimodt-Moller, A.S. Cohen, Simultaneous quantification of isoniazid, rifampicin, ethambutol and pyrazinamide by liquid chromatography/tandem mass spectrometry, APMIS 124 (2016) 1004–1015.
[15] M.T. Chirehwa, H. McIlleron, L. Wiesner, D. Affolabi, O. Bah-Sow, C. Merle, P. Denti, R. team, Effect of efavirenz-based antiretroviral therapy and high-dose rifampicin on the pharmacokinetics of isoniazid and acetyl-isoniazid, J. Antimicrob. Chemother. 74 (2019) 139–148.
[16] S.K. Heysell, J.L. Moore, S.J. Keller, E.R. Houpt, Therapeutic drug monitoring for slow response to tuberculosis treatment in a state control program, Virginia, USA, Emerg. Infect. Dis. 16 (2010) 1546–1553.
[17] A. Babalik, A. Babalik, S. Mannix, D. Francis, D. Menzies, Therapeutic drug monitoring in the treatment of active tuberculosis, Canc. Res. J. 18 (2011) 225–229.
[18] J.G. Pasipanodya, H. McIlleron, A. Burger, P.A. Wash, P. Smith, T. Gumbo, Serum drug concentrations predictive of pulmonary tuberculosis outcomes, J. Infect. Dis. 208 (2013) 1464–1473.
[19] 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.
[20] M. Han, S.H. Jun, J.H. Lee, K.U. Park, J. Song, S.H. Song, Method for simultaneous analysis of nine second-line anti-tuberculosis drugs using UPLC-MS/MS, J. Antimicrob. Chemother. 68 (2013) 2066–2073.
[21] D.H. Vu, R.A. Koster, A.M. Wessels, B. Greijdanus, J.W. Alffenaar, D.R. Uges, Troubleshooting carry-over of LC-MS/MS method for rifampicin, clarithromycin and metabolites in human plasma, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 917–918 (2013) 1–4.
[22] A. Srivastava, D. Waterhouse, A. Ardrey, S.A. Ward, Quantification of rifampicin in human plasma and cerebrospinal fluid by a highly sensitive and rapid liquid

chromatographic-tandem mass spectrometric method, J. Pharmaceut. Biomed. Anal. 70 (2012) 523–528.
[23] 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. Pharmaceut. Biomed. Anal. 102 (2015) 253–260.
[24] 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. Pharmaceut. Biomed. Anal. 102 (2015) 9–16.
[25] L.A. Moussa, C.E. Khassouani, R. Soulaymani, M. Jana, G. Cassanas, R. Alric, B. Hue, Therapeutic isoniazid monitoring using a simple high-performance liquid chromatographic method with ultraviolet detection, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 766 (2002) 181–187.
[26] S.L. Beal, Ways to fit a PK model with some data below the quantification limit, J. Pharmacokinet. Pharmacodyn. 28 (2001) 481–504.
[27] S. Chideya, C.A. Winston, C.A. Peloquin, W.Z. Bradford, P.C. Hopewell, C.D. Wells, A.L. Reingold, T.A. Kenyon, T.L. Moeti, J.W. Tappero, Isoniazid, rifampin, ethambutol, and pyrazinamide pharmacokinetics and treatment outcomes among a predominantly HIV-infected cohort of adults with tuberculosis from Botswana, Clin. Infect. Dis. 48 (2009) 1685–1694.
[28] A. Daskapan, L.R. Idrus, M.J. Postma, B. Wilffert, J.G.W. Kosterink, Y. Stienstra, D.J. Touw, A.B. Andersen, A. Bekker, P. Denti, A.K. Hemanth Kumar, K. Jeremiah, A. Kwara, H. McIlleron, G. Meintjes, J.J. van Oosterhout, G. Ramachandran, N. Rockwood, R.J. Wilkinson, T.S. van der Werf, J.C. Alffenaar, A systematic review on the effect of HIV infection on the pharmacokinetics of first-line tuberculosis drugs, Clin. Pharmacokinet. 58 (2019) 747–766.
[29] P. Gurumurthy, G. Ramachandran, A.K. Hemanth Kumar, S. Rajasekaran, C. Padmapriyadarsini, S. Swaminathan, S. Bhagavathy, P. Venkatesan, L. Sekar, A. Mahilmaran, N. Ravichandran, P. Paramesh, Decreased bioavailability of rifampin and other antituberculosis drugs in patients with advanced human immunodeficiency virus disease, Antimicrob. Agents Chemother. 48 (2004) 4473–4475.
[30] G. Ramachandran, A.K. Kumar, T. Kannan, P.K. Bhavani, S.R. Kumar, N.P. Gangadevi, V.V. Banurekha, V. Sudha, S. Venkatesh, N. Ravichandran, S. Kalpana, G. Mathevan, G.N. Sanjeeva, D. Agarwal, S. Swaminathan, Low serum concentrations of rifampicin and pyrazinamide associated with poor treatment outcomes in children with tuberculosis related to HIV status, Pediatr. Infect. Dis. J. 35 (2016) 530–534.
[31] M. Zhu, W.J. Burman, J.R. Starke, J.J. Stambaugh, P. Steiner, A.E. Bulpitt, D. Ashkin, B. Auclair, S.E. Berning, R.W. Jelliffe, G.S. Jaresko, C.A. Peloquin, Pharmacokinetics of ethambutol in children and adults with tuberculosis, Int. J. Tubercul. Lung Dis. 8 (2004) 1360–1367.
[32] H. Yang, A. Enimil, F.S. Gillani, S. Antwi, A. Dompreh, A. Ortsin, E. Adu Awhireng, M. Owusu, L. Wiesner, C.A. Peloquin, A. Kwara, Evaluation of the adequacy of the 2010 revised world Health organization recommended dosages of the first-line antituberculosis drugs for children: adequacy of revised dosages of TB drugs for children, Pediatr. Infect. Dis. J. 37 (2018) 43–51.
[33] D.P. Holland, C.D. Hamilton, A.C. Weintrob, J.J. Engemann, E.R. Fortenberry, C.A. Peloquin, J.E. Stout, Therapeutic drug monitoring of antimycobacterial drugs in patients with both tuberculosis and advanced human immunodeficiency virus infection, Pharmacotherapy 29 (2009) 503–510.
[34] A. Alsultan, C.A. Peloquin, Therapeutic drug monitoring in the treatment of tuberculosis: an update, Drugs 74 (2014) 839–854.
[35] Q. Lei, H. Wang, Y. Zhao, L. Dang, C. Zhu, X. Lv, H. Wang, J. Zhou, Determinants of serum concentration of first-line anti-tuberculosis drugs from China, Medicine (Baltim.) 98 (2019), e17523.
[36] R.K. Verbeeck, G. Gunther, D. Kibuule, C. Hunter, T.W. Rennie, Optimizing treatment outcome of first-line anti-tuberculosis drugs: the role of therapeutic drug monitoring, Eur. J. Clin. Pharmacol. 72 (2016) 905–916.
[37] M. Zhu, J.R. Starke, W.J. Burman, P. Steiner, J.J. Stambaugh, D. Ashkin, A.E. Bulpitt, S.E. Berning, C.A. Peloquin, Population pharmacokinetic modeling of pyrazinamide in children and adults with tuberculosis, Pharmacotherapy 22 (2002) 686–695.
[38] B. Dasht Bozorg, A. Goodarzi, F. Fahimi, P. Tabarsi, N. Shahsavari, F. Kobarfard, F. Dastan, Simultaneous determination of isoniazid, pyrazinamide and rifampin in human plasma by high-performance liquid chromatography and UV detection, Iran. J. Pharm. Res. 18 (2019) 1735–1741.
[39] G. Ramachandran, A.K. Kumar, P.K. Bhavani, T. Kannan, S.R. Kumar, N.P. Gangadevi, V.V. Banurekha, L. Sekar, N. Ravichandran, G. Mathevan, G.N. Sanjeeva, R. Dayal, S. Swaminathan, Pharmacokinetics of first-line antituberculosis drugs in HIV-infected children with tuberculosis treated with intermittent regimens in India, Antimicrob. Agents Chemother. 59 (2015) 1162–1167.
[40] 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 (Edinb). 109 (2018) 28–34.
[41] 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.
[42] N. Maggi, C.R. Pasqualucci, R. Ballotta, P. Sensi, Rifampicin: a new orally active rifamycin, Chemotherapy 11 (1966) 285–292.
[43] A. Ghosh, S. Ganguly, N. Dey, S. Banerjee, A. Das, D.J. Chattopadhyay, I.B. Chatterjee, Causation of cigarette smoke-induced emphysema by p-benzoquinone and its prevention by vitamin C, Am. J. Respir. Cell Mol. Biol. 52 (2015) 315–322.
[44] N. Vahdati-Mashhadian, M.R. Jafari, N. Sharghi, T. Sanati, Protective effects of vitamin C and NAC on the toxicity of rifampin on Hepg2 cells, Iran. J. Pharm. Res. 12 (2013) 141–146.
[45] E. Ribera, C. Azuaje, R.M. Lopez, P. Domingo, A. Curran, M. Feijoo, L. Pou, P. Sanchez, M.A. Sambeat, J. Colomer, J.L. Lopez-Colomes, M. Crespo, V. Falco, I. Ocana, A. Pahissa, Pharmacokinetic interaction between rifampicin and the once-daily combination of saquinavir and low-dose ritonavir in HIV-infected patients with tuberculosis, J. Antimicrob. Chemother. 59 (2007) 690–697.
[46] F. Roblot, J.M. Besnier, B. Giraudeau, N. Simonnard, A.P. Jonville-Bera, P. Coipeau, P. Choutet, E. Autret-Leca, C. Le Guellec, Lack of association between rifampicin plasma concentration and treatment-related side effects in osteoarticular infections, Fundam. Clin. Pharmacol. 21 (2007) 363–369.