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Analyzing the Effect of Resveratrol on Pharmacokinetics of Antituberculosis Drug Bedaquiline in Rats by a Novel UPLC-MS/MS Approach

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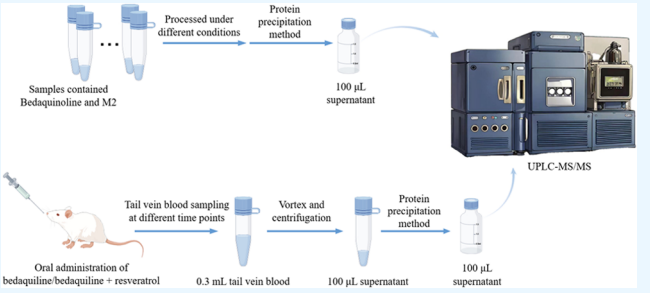
ABSTRACT: Bedaquiline (BDQ), a diarylquinoline compound, is an inhibitor of mycobacterial ATP synthase, specifically with FDA approval as a treatment for multidrug-resistant tuberculosis (MDR-TB). M2 is the main metabolite of BDQ and is active against tuberculosis. The objective of this study was to establish and validate a sensitive and convenient ultraperformance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) approach to concurrently quantify BDQ and M2 in rat plasma and to examine whether resveratrol, a CYP3A4 inhibitor, could influence the pharmacokinetics of BDQ and M2 in rats. Plasma samples containing the internal standard (IS) linezolid were formulated by adding acetonitrile for a simple one-step protein precipitation, and the analytes in samples were quantified by the UPLC-MS/MS method. BDQ and M2 were successfully calibrated in the ranges of 0.5−1000 and 1.0−200 ng/mL, where the lower limit of quantification (LLOQ) was 0.5 and 1.0 ng/mL, respectively. The precisions and accuracies of BDQ and M2 were in compliance with the FDA analytical standards. Recoveries and matrix effects of the analytes were satisfactory, and the analytes remained stable under four different temperatures and conditions. The well-validated UPLC-MS/MS method was successfully applied to the study of the food-drug interaction in rats. Remarkably, resveratrol increased the level of exposure of BDQ. Furthermore, the effect of resveratrol on the metabolism of BDQ and M2 needs further clinical studies.



1. INTRODUCTION

Multidrug-resistant tuberculosis (MDR-TB) is an infection in which *Mycobacterium tuberculosis* (TB) is resistant to at least rifampin and isoniazid.[1] Second-line antituberculosis drugs are recommended for the treatment of MDR-TB, which usually costs 18−24 months.[2] Bedaquiline (BDQ) is an FDA-approved drug for treating MDR-TB and is one of the second-line antituberculosis drugs.[3] BDQ is a diarylquinoline compound that is specifically able to inhibit mycobacterial ATP synthase.[4] It was found that in a randomized phase II placebo-controlled clinical trial, patients with MDR-TB who received the standard MDR regimen and BDQ therapy converted to negative cultures within 8 weeks in 48%, versus 9% in the placebo group receiving only the standard MDR regimen.[5] After 24 weeks, the proportions of successfully treated patients in the BDQ and placebo groups were 78.8 and 57.6%, respectively.[5] Nevertheless, QT interval prolongation could be caused by BDQ, which might result in irregular and fatal heart rhythms.[6] In addition, other adverse reactions, such as drug-related hepatic disorders, metabolism and nutrition disorders (hyperuricemia being the most common), electrolyte disorders, and gastrointestinal system disorders can also occur during the treatment of BDQ.[7]

The description of BDQ metabolism will be valuable for the prediction and prevention of BDQ-related drug- or food-drug interactions. M2, the N-monodes-methyl metabolite, is one of the main products of BDQ which is metabolized in the liver by cytochrome P450 (CYP) isoenzyme 3A4.[8] M2 is 3 to 6 times less active against tuberculosis than BDQ.[9] Studies have shown that the cytotoxicity and phospholipidogenic potential of M2 was greater than that of BDQ.[9] Moreover, concentrations of M2 have also been associated with QT-prolongation.[10] Therefore, it is necessary to describe the metabolic process of M2 *in vivo*[11] Drug-drug and food-drug interactions related to CYP3A4 could strongly affect plasma or tissue drug concentrations and therefore lead to serious toxic effects.

Resveratrol (3,5,4′-trihydroxystilbene, RVT) is a polyphenol phytochemical that is a common ingredient in many plants or beverages (e.g., berries, grapes, peanuts, soybeans), wine,

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cranberry and grape juice, and many other plant products.[12,13] Because of the wide range of pharmacological effects of RVT,[14−17] it has been ingested as a dietary supplement. Investigations have shown that RVT can inhibit the activity of CYP3A4.[16,18] High intake of RVT could theoretically increase the bioavailability and toxicity risk of drugs by inhibiting intestinal CYP3A4 and reducing the first-pass effect.[16] As a consequence, there is a risk of food-drug interactions in patients who ingest large doses of RVT in combination with other drugs metabolized by CYP3A4.

In this study, the pharmacokinetics of BDQ and M2 were investigated in rats with an ultraperformance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method. Best of our knowledge, no data are available concerning the impacts of RVT on the pharmacokinetics of BDQ and M2. Therefore, this study was conducted to research the effect of RVT on the pharmacokinetics of BDQ and M2 in rats. The results may be useful for the evaluation of interactions in BDQ, M2, and RVT in the clinic.

2. EXPERIMENTAL SECTION

2.1. Chemicals and Materials. BDQ (purity >98%), M2 (purity >98%), linezolid (used as an internal standard, IS, purity >98%), and RVT (purity >98%) were obtained from Beijing sunflower Technology Development Co., Ltd. (Beijing, China). Analytical grade of formic acid was also purchased from Beijing Sunflower and Technology Development Co., Ltd. Methanol (LC-MS grade) and acetonitrile (LC-MS grade) were acquired from Merck (Darmstadt, Germany). Sodium carboxymethyl cellulose (CMC-Na) was sourced from Canspec Scientific Instruments Co., Ltd. (Shanghai, China). The ultrapure water used to prepare the solutions, and the mobile phase was produced by Milli-Q purification (Millipore, Bedford).

2.2. UPLC-MS/MS Conditions. Samples were analyzed using a Waters ACQUITY UPLC I-Class system (Milford, MA) coupled to a Waters Xevo TQ-S triple quadrupole mass spectrometer. Chromatographic separation was conducted at 40 °C using gradient elution with different ratios of 0.1% formic acid aqueous solution (A)-acetonitrile (B) on a UPLC BEH C18 (2.1 × 50 mm, 1.7 μm) column. After the mobile phase ratio was optimized several times, the gradient elution procedure was adjusted as follows: 0−0.5 min (B, set at 10%), 0.5−1.0 min (B, linearly increased to 90%), 1.0−1.4 min (B, maintained at 90%), and 1.4−1.5 min (B, quickly decreased to 10%). Finally, at 1.5 and 2.0 min, 10% acetonitrile was equilibrated for the baseline. The temperature of theautosampler was controlled at 10 °C during the running of sequences with the flow rate of 0.3 mL/min.

With the electrospray ionization (ESI) interface set to positive ion mode, positively charged ions were collected. Through the precursor ion scan and the product ion scan, the strong and stable $[M + H]^+$ ions and their corresponding daughter ions were identified. The chemical structure and mass spectra of BDQ, M2 as well as IS were described in Figure 1. In multiple reaction monitoring (MRM) mode, the quantitative analysis was performed following one selective transition for each compound with m/z 555.00 → 58.08 for BDQ, m/z 540.93 → 479.92 for M2, and m/z 338.01 → 296.03 for IS (shown in Table 1). For BDQ, M2, and IS, the collision energy was 20, 15, and 15 eV, respectively. The cone voltage of each analyte was 30 V. The selected parameters of MS were: 1000 °C desolvation gas temperature, 2.0 kV capillary voltage, 200 L/h cone gas, 0.15 mL/min collision gas, and 1000 L/h desolvent gas. The acquisition and analysis of all data were carried out using Masslynx 4.1 software (Waters Corp., Milford, MA).

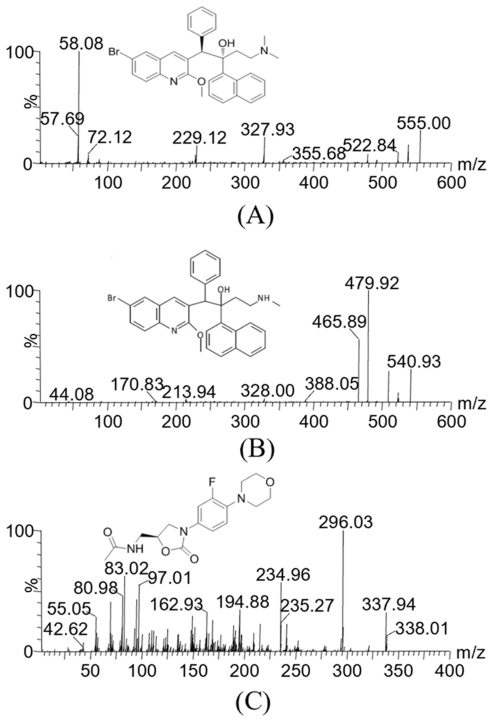

Figure 1. Chemical structures and mass spectra of BDQ (A), M2 (B), and linezolid (IS, C) in the present study.

Table 1. Specific Mass Spectrometric Parameters and Retention Times (RTs) for the Analytes and IS, Including Cone Voltage (CV) and Collision Energy (CE)

analytes	precursor ion	product ion	CV (V)	CE (eV)	RT (min)
BDQ	555.00	58.08	30	20	1.62
M2	540.93	479.92	30	15	1.61
IS	338.01	296.03	30	15	1.42

2.3. Solution Preparation. Initial stock solutions of 100 μg/mL were prepared in methanol by dissolving accurately weighed BDQ, M2 and IS standard solutions, respectively. A range of working solutions used to construct quality control samples (QCs) and calibration curves were obtained by gradually diluting stock solutions of BDQ and M2 with methanol.

Preparations were made for four levels of QCs, which included high (HQC), medium (MQC), low (LQC), and lower limit of quantification (LLOQ) concentrations. The IS working solution was also obtained by using methanol as a diluent. The IS working solution (200 ng/mL) remained unchanged during the entire methodological validation. All primary stock solutions were preserved at −80 °C prior to use.

2.4. Plasma Sample Treatment. In a newly labeled centrifuge tube, a 100 μL plasma sample was mixed with 20 μL of IS solution and 300 μL of acetonitrile. Subsequently, the whole mixture was vortexed briefly for 1.0 min. After 10 min of centrifugation (13,000*g*, 4 °C), the 100 μL supernatant was pipetted into a new autosampler vial. The injection volume of UPLC/MS-MS was set to 2 μL during the running of the samples.

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2.5. Methodological Validation. The analytical method in this current experiment was fully validated based on the FDA guidelines.[19] Sensitivity, linearity, selectivity, accuracy, precision, matrix effect, extraction recovery, and stability of the developed method were investigated as follows.

2.5.1. Selectivity. Blank plasma, blank plasma samples with added analyte and IS, and actual plasma samples administered orally with BDQ and M2 were compared to evaluate the selectivity of the method (n = 6). The analytical method was found to have good selectivity because interference from matrix components or other substances did not influence the retention time of each analyte.

2.5.2. Sensitivity and Linearity. Concentrations of calibrated curves for BDQ and M2 were 0.5−1000 and 1.0−200 ng/mL, respectively. Each calibrated curve containing 8 points was used to assess the linearity of sample concentrations and response values. The ratios of the peak area of each analyte to the IS versus the plasma concentration were employed to generate the calibration curve. The linear equation was constructed using a weighted ($1/x^2$) least-squares regression method. The correlation coefficient (r^2) supposed to be >0.99 was used to evaluate the linear relationship. The concentrations were obtained by back-calculation based on the peak area.

LLOQ was the minimum concentration of the calibration curve that had a reasonable precision (RSD%) below 20% and an accuracy (RE%) between 80 and 120%.

2.5.3. Accuracy and Precision. Assessments of intraday and interday precision and accuracy were performed by six-tuple analysis of QCs on three consecutive days. There were four QC levels including 0.5, 1, 80, and 800 ng/mL as LLOQ, LQC, MQC, and HQC for BDQ, and 1.0, 2.0, 40, and 160 ng/mL for M2, respectively. According to the FDA analytical standards, the accuracies of QC concentration (except LLOQ) calculated from the calibration curve should be less than ±15% of its nominal level and precisions were within 15%.

2.5.4. Extraction recovery and Matrix Effect. Assessment of extraction recovery for each analyte was conducted by comparing processed QCs versus extracted blank plasma with the addition of analytes. Matrix effects were assessed by comparing the peak areas of analytes added to extracted plasma samples at QCs (HQC, MQC, and LQC) versus standard solutions of corresponding concentrations (n = 6).

2.5.5. Stability. Similarly, the stability of the analytes in rat plasma was examined by using three concentrations of QCs. It was carried out including stability at room temperature (2 h storage at room temperature), stability after preparation (5 h storage in an autosampler at 10 °C), stability in the long term (21 days at −80 °C), and frozen-thaw stability (three repeated freeze−thaw cycles). Replications of each QC were performed 5 times for analysis, and the deviations were calculated to demonstrate the stability.

2.6. Animals and Food-Drug Interaction. Ten healthy male Sprague−Dawley rats (SD rats, weight 190 ± 10 g) for the pharmacokinetic study were purchased from the Laboratory Animal Center of the First Affiliated Hospital of Wenzhou Medical University (Zhejiang, China), and the animal experimental operations were approved by the Animal Care Committee of the First Affiliated Hospital of Wenzhou Medical University (Zhejiang, China). First, the rats were housed for 1 week in an environment with adequate light, water, and food for acclimatization. Animals were then fasted for 12 h prior to gavage administration, but water intake was not restricted. BDQ and RVT were administered at a dosage that was one-tenth of the standard daily human dose. For studying the effect of RVT on the pharmacokinetic of BDQ and M2, ten SD rats were divided into two groups: 20 mg/kg BDQ alone (group A, n = 5) and 20 mg/kg BDQ with the combination of 50 mg/kg RVT group (group B, n = 5).[20,21] The drugs were suspended in 0.5% carboxymethyl cellulose (CMC) solution. RVT was given by gavage half an hour before BDQ administration. Approximately 0.3 mL of rat tail vein blood was collected at 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after oral administration, placed in heparin-containing tubes, and centrifuged at 4000 rpm for 8 min immediately. Afterward, the supernatant was transferred to a new tube and saved at −80 °C to be analyzed.

The pharmacokinetics of the analytes were evaluated from the plasma concentration−time data of BDQ and M2 of each animal, and the main parameters were calculated using the noncompartment method by the Drug and Statistics Software (DAS). The maximum plasma concentration (C_{max}) and the time to reach C_{max} (t_{max}) could be obtained directly from the data. While the area under the plasma concentration−time curve (AUC_{0-t}) could be calculated, and $AUC_{0-\infty}$ was the extended area extrapolated from the predicted and quantifiable concentrations at the last time point. $t_{1/2}$ refers to the elimination of half-life. Apparent clearance (CLz/F) was the ratio of dose to area under the plasma concentration−time curve. The independent samples t-test for pharmacokinetic parameters was performed using the Statistical Package for Social Sciences (SPSS, version 18.0, Chicago, IL) in order to assess the interactions of RVT on BDQ and M2, and a p-value <0.05 was regarded as significantly different.

3. RESULTS AND DISCUSSION

3.1. UPLC−MS/MS Method Conditions. In the current study, we established a bioanalytical method with high sensitivity and reliability for the simultaneous determination of BDQ and M2 in rat plasma by UPLC-MS/MS. The compounds were easily protonated on amino groups; therefore, MRM was selected for positive monitoring mode. The $[M + H]^+$ of BDQ, M2, and IS were m/z 555.00, 540.93, and 338.01 (as exhibited in Figure 1), and the most abundant product ions were m/z 58.08, 479.92, and 296.03, respectively. As a consequence, the mother-to-daughter of quantifier conversions were m/z 555.00 → 58.08 for BDQ, m/z 540.93 → 479.92 for M2, and m/z 338.01 → 296.03 for IS, respectively.

For the purposes of achieving desirable separations, symmetrical peak shapes, and shorter retention time, as well as minimizing matrix effects, the chromatographic conditions (mobile phase, analytical column, flow rate, and gradient elution) were optimized. Acetonitrile was used as the organic phase on the basis that it provided a flatter baseline and better quantitative results. With regard to the aqueous phase, when 0.1% formic acid aqueous solution was used, it facilitated the positive ionization of the analytes, obtaining favorable peak shapes and a shorter retention time.

It was also optimized for mass parameter conditions to obtain a better resolution and higher response. At the end of MS/MS optimization, the signal of the fragment with the highest abundance was used for quantification (presented in Table 1).

3.2. Plasma Sample Treatment. In the preparation of plasma samples, protein precipitation (PPT) is an easy-

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operated method. The use of organic solvents for PPT allowed the removal of large amounts of plasma proteins rapidly with little pollution and less cost. It has been shown from previous experiences that the sample processing using acetonitrile for PPT was short and the recovery was high.[22,23] Therefore, acetonitrile was used for the precipitation of plasma proteins in the current sample processing.

3.3. Validation of Methodology. *3.3.1. Selectivity.* Typical chromatograms for assessing selectivity are shown in Figure 2, indicating that there were no endogenous substances

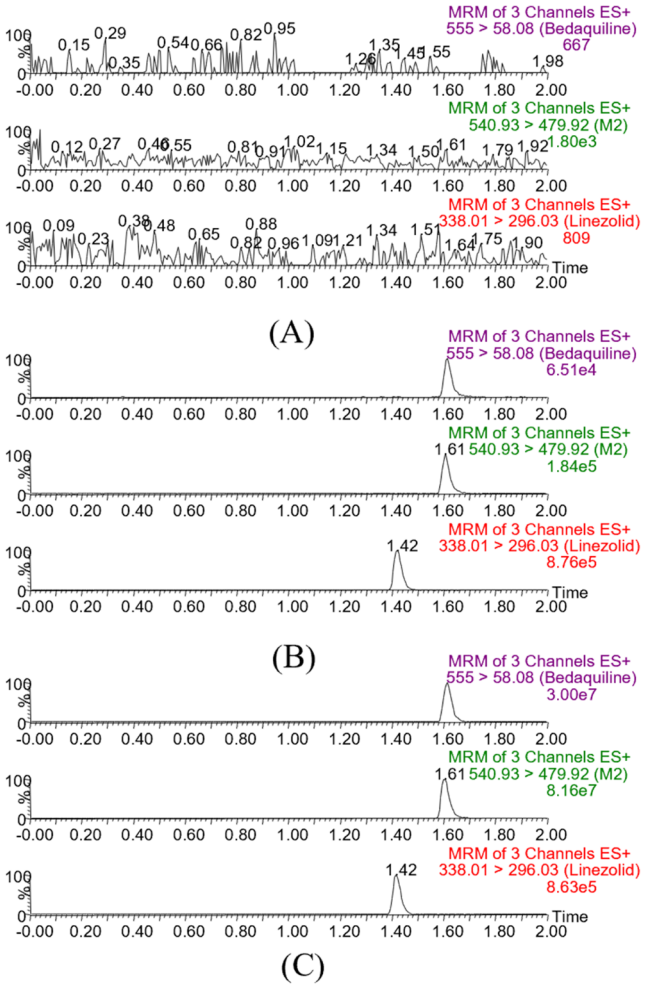

Figure 2. Representative MRM chromatograms of BDQ, M2 and IS in SD rat sample: blank plasma (A), blank plasma spiked with standard solutions (B) and real plasma sample collected from a rat following oral administration of 20 mg/kg BDQ after 1 h (C).

interfering. It was found that the retention time of BDQ, M2 and IS was 1.62, 1.61, and 1.42 min, respectively, demonstrating the high selectivity of the analytical method.

3.3.2. Linearity and Sensitivity. For the calibration curves, there were excellent linearities in the ranges of 0.5−1000 ng/mL for BDQ and 1.0−200 ng/mL for M2 (as demonstrated in Table 2). The validated linear regression equations were as follows: $Y = 4.99893X + 0.399759$ (r^2= 0.997, BDQ) and $Y = 28.072X + 2.81722$ (r^2= 0.999, M2). The LLOQ values of BDQ and M2 were 0.5 and 1 ng/mL, respectively, and the samples showed identifiable peaks with sufficient sensitivity for pharmacokinetic studies of BDQ and M2.

Table 2. Calibration Curves for the Analytes of BDQ and M2 in SD Rat Plasma

analytes	regression equation	r^2	linear range (ng/mL)	LLOQ (ng/mL)
BDQ	$y = 4.99893x + 0.399759$	0.997	0.5−1000	0.5
M2	$y = 28.072x + 2.81722$	0.999	1.0−200	1

3.3.3. Precision and Accuracy. The developed method was validated over three consecutive days. An accurate and precise summary of the LLOQs and QCs were presented in Table 3.

Table 3. Accuracy and Precision of Each Analyte in SD Rat Plasma (n = 6)

		intraday		interday	
analytes	concentration (ng/mL)	RSD (%)	RE (%)	RSD (%)	RE (%)
BDQ	0.5	12.2	2.1	11.6	6.2
	1.0	7.3	−13.8	9.2	−14.0
	80	2.3	3.8	6.7	8.6
	800	3.3	−13.4	3.2	−12.0
M2	1.0	11.1	−9.0	11.2	−8.1
	2.0	7.0	−8.0	9.2	−3.7
	4.0	1.5	5.4	7.1	12.0
	160	2.8	4.7	2.9	5.3

Intraday and interday precision of the three QCs and LLOQ was found to be less than 12.2%. And the accuracy of the analytes did not exceed ±14.0%. This present method achieved good data reproducibility, as all data were within the acceptable validation criteria for the compounds.

3.3.4. Matrix Effect and Recovery. High extraction recoveries (>75%) were obtained for each analyte, as shown in Table 4. Moreover, the data of matrix effect ranged from

Table 4. Recovery and Matrix Effect of Each Analyte in SD Rat Plasma (n = 6)

		recovery (%)		matrix effect (%)	
analytes	concentration (ng/mL)	mean ± SD	RSD (%)	mean ± SD	RSD (%)
BDQ	1.0	76.5 ± 4.2	5.5	96.8 ± 13.0	13.4
	80	76.8 ± 2.7	3.5	101.6 ± 1.6	1.6
	800	77.3 ± 1.6	2.0	92.5 ± 2.5	2.7
	2.0	87.3 ± 6.4	7.3	114.6 ± 14.1	12.3
M2	40	89.0 ± 3.4	3.9	106.9 ± 5.7	5.4
	160	91.2 ± 4.9	5.3	112.8 ± 4.3	3.9

92.5 to 114.6% for all analytes, indicating negligible ion enhancement or suppression in rat plasma was observed under the developed analytical conditions of BDQ, M2 and IS. The recoveries and matrix effects met the requirements for methodological validation, demonstrating the measurability of this analytical method was good.

3.3.5. Stability. The results of the stability assay were presented in Table 5. It was revealed that BDQ and M2 were stable at four different temperatures and conditions.

3.4. Food-Drug Interaction. The currently developed UPLC-MS/MS approach has been used to quantitatively determine the plasma concentrations of BDQ and M2 in rats after a single oral dose of 20 mg/kg BDQ alone or combined with 50 mg/kg RVT. Mean plasma concentration−time curves of groups A and B are shown in Figure 3. The main

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Table 5. Stability Results of Each Analyte in Rat Plasma under Different Conditions (n = 5)

		room temperature, 2 h		autosampler 10 °C, 5 h		three freeze−thaw		−80 °C, 21 days	
analytes	concentration (ng/mL)	RSD (%)	RE (%)	RSD (%)	RE (%)	RSD (%)	RE (%)	RSD (%)	RE (%)
BDQ	1.0	13.5	−1.6	9.3	6.9	13.3	−8.7	14.5	−14.4
	80	4.7	6.2	6.8	14.3	2.6	−0.6	3.6	−4.9
	800	3.2	−0.3	3.7	−12.8	3.9	−7.9	3.8	−8.4
M2	2.0	9.5	−6.6	9.7	−3.2	7.7	−0.8	6.4	−4.5
	40	6.4	8.1	8.6	13.5	4.0	7.4	3.7	2.1
	160	4.2	10.1	4.6	11.0	4.2	−0.3	4.1	−2.8

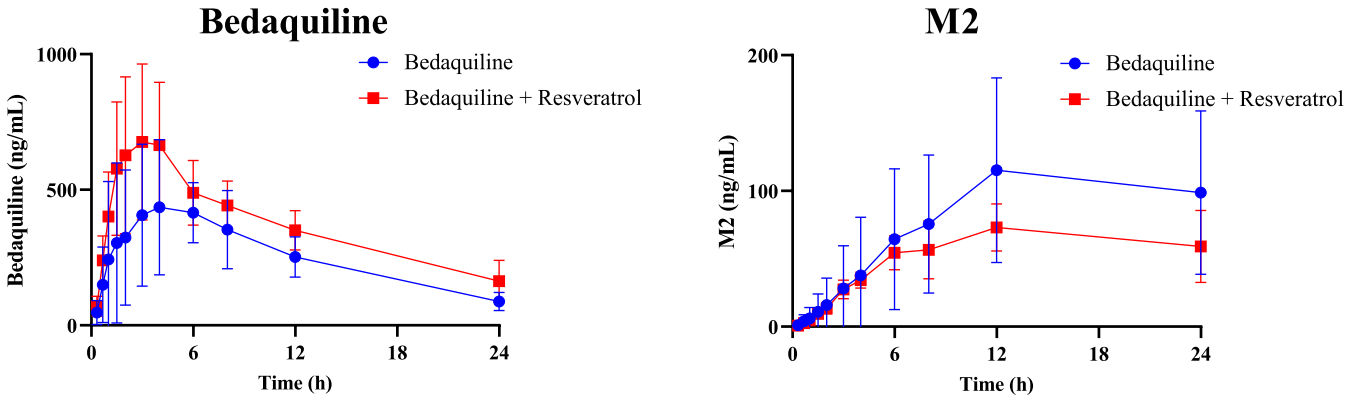

Figure 3. Mean plasma concentration−time curves of BDQ and M2 in male SD rats after oral administration of 20 mg/kg BDQ alone or with 50 mg/kg resveratrol (n = 5, Mean ± SD).

pharmacokinetic parameters of BDQ and M2 were concluded in Tables 6 and 7, with the values expressed as mean ± standard deviation (SD).

Table 6. Main Pharmacokinetic Parameters of BDQ in SD Rats after Orally Administrated 20 mg/kg BDQ Alone and Combined with 50 mg/kg Resveratrol in SD Rats (n = 5, Mean ± SD)

parameters	BDQ	BDQ + resveratrol
$AUC_{0\to t}$ (ng/mL·h)	6050.75 ± 1997.91	8789.61 ± 1387.01[a]
$AUC_{0\to\infty}$ (ng/mL·h)	7160.24 ± 2368.25	11708.62 ± 3223.98[a]
$t_{1/2}$ (h)	8.36 ± 2.50	11.18 ± 3.37
T_{max} (h)	5.50 ± 2.40	3.60 ± 1.52
CLz/F (L/h/kg)	3.07 ± 1.05	1.82 ± 0.50[a]
C_{max} (ng/mL)	530.06 ± 193.37	771.64 ± 217.22

[a]p < 0.05, significant in comparison to rats dosed with BDQ alone.

Table 7. Main Pharmacokinetic Parameters of M2 in SD Rats after Orally Administrated of 20 mg/kg BDQ Alone and Combined with 50 mg/kg Resveratrol in SD Rats (n = 5, Mean ± SD)[a]

parameters	BDQ	BDQ + resveratrol
$AUC_{0\to t}$ (ng/mL·h)	1976.23 ± 1266.28	1313.64 ± 398.41
$AUC_{0\to\infty}$ (ng/mL·h)	6204.78 ± 3330.23	3919.04 ± 1119.79
$t_{1/2}$ (h)	7.54 ± 4.44	10.29 ± 5.95
T_{max} (h)	12.00 ± 0.00	14.40 ± 5.37
CLz/F (L/h/kg)	3.91 ± 1.75	5.46 ± 1.60
C_{max} (ng/mL)	115.20 ± 68.10	73.45 ± 18.29

[a]p < 0.05, significant in comparison to rats dosed with BDQ alone.

Compared with BDQ dosed alone, $AUC_{0\to t}$ and $AUC_{0\to\infty}$ of BDQ were significantly increased, and CLz/F was markedly decreased in combination oral of RVT with the p-value <0.05, suggesting that RVT could increase the oral bioavailability of BDQ. No statistical differences were found for other pharmacokinetic parameters, probably owe to the finite number of animals. In addition, there were no significant changes in the pharmacokinetic parameters of M2 in the oral RVT group compared with the BDQ-only group.

According to previous reports, RVT could influence the pharmacokinetics of drugs by inhibiting the CYP3A4. For example, RVT could increase the AUC of buspirone by 1.33-fold in humans,[24] C_{max} and AUC of nicardipine by 2.2 and 2.3-fold,[25] AUC and C_{max} of diltiazem by 1.6-fold in rats.[26] These drugs were the substrates of CYP3A4. In our study, RVT could increase the $AUC_{0\to t}$ and $AUC_{0\to\infty}$ of BDQ. There was a possibility that RVT inhibited CYP3A4 in the intestine, decreasing the first-pass effect of BDQ and thus increasing the bioavailability. Therefore, exposure to BDQ would be significantly increased, and its associated side effects would be more serious and frequent. It has also been reported that therapy drug monitoring (TDM) can be used for patients taking oral antituberculosis drugs to individualize dosing for reducing adverse effects.[27] Given the widespread availability of RVT, the combination of BDQ and RVT should be monitored in the clinic. Finally, further studies about the effect of RVT on BDQ metabolism in clinical studies and its inhibitory mechanism should be further investigated.

4. CONCLUSIONS

In the current study, a UPLC-MS/MS method with sensitivity and rapidity was developed for simultaneously determining BDQ and M2 in the plasma of SD rats. It was economical and rapid in sample preparation with a short retention time and achieved low LLOQ values for BDQ and M2. Subsequently, the method was well employed for pharmacokinetic studies in rats. In addition, by administering RVT orally 0.5 h earlier, the effect of food-drug interaction on the pharmacokinetic

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behavior of BDQ and M2 was investigated. The results indicated that there was potential inhibition of RVT on the metabolism of BDQ, which contributes to individualized dosing of BDQ clinically. Also, the findings could be a valuable reference for other drugs with similar properties.

■ ASSOCIATED CONTENT

Data Availability Statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

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Author Contributions

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Notes

The authors declare no competing financial interest.

Animal experiments were demonstrated to be ethically acceptable and were carried out according to the Guidelines of the Experimental Animal Care and Use of Laboratory Animals of The First Affiliated Hospital of Wenzhou Medical University. All animal procedures and experimental protocols were approved by the Laboratory Animal Ethics Committee of The First Affiliated Hospital of Wenzhou Medical University (Ethics approval number: WYYY-IACUC-AEC-2023−046).

■ REFERENCES

(1) Chang, K. C.; Yew, W.-W. Management of difficult multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis: Update 2012. *Respirology* **2012**, *18*, 8−21, DOI: 10.1111/j.1440-1843.2012.02257.x.

(2) Mukherjee, J. S.; Rich, M. L.; Socci, A. R.; Joseph, J. K.; Virú, F.; Shin, S. S.; Furin, J. J.; Becerra, M. C.; Barry, D. J.; Kim, J. Y. J. L. Programmes and principles in treatment of multidrug-resistant tuberculosis. *Lancet* **2004**, *363* (9407), 474−481.

(3) Cox, E.; Laessig, K. FDA approval of bedaquiline–the benefit-risk balance for drug-resistant tuberculosis. *N. Engl. J. Med.* **2014**, *371* (8), 689−691.

(4) Cole, S. T.; Alzari, P. M. TB–A New Target, a New Drug. *Science* **2005**, *307* (5707), 214−215, DOI: 10.1126/science.1108379.

(5) Diacon, A. H.; Donald, P. R.; Pym, A.; Grobusch, M.; Patientia, R. F.; Mahanyele, R.; Bantubani, N.; Narasimooloo, R.; Marez, T. D.; van Heeswijk, R.; et al. Randomized Pilot Trial of Eight Weeks of Bedaquiline (TMC207) Treatment for Multidrug-Resistant Tuberculosis: Long-Term Outcome, Tolerability, and Effect on Emergence of Drug Resistance. *Antimicrob. Agents Chemother.* **2012**, *56* (6), 3271−3276.

(6) Isralls, S.; Baisley, K.; Ngam, E.; Grant, A. D.; Millard, J. QT interval prolongation in people treated with bedaquiline for drug-resistant tuberculosis under programmatic conditions: a retrospective cohort study. *Open Forum Infect. Dis.* **2021**, *8*, No. ofab413, DOI: 10.1093/ofid/ofab413.

(7) Skrahina, A.; Hurevich, H.; Falzon, D.; Zhilevich, L.; Rusovich, V.; Dara, M.; Setkina, S. Bedaquiline in the multidrug-resistant tuberculosis treatment: Belarus experience. *Int. J. Mycobact.* **2016**, *5* (5), S62−S63, DOI: 10.1016/j.ijmyco.2016.11.014.

(8) Liu, K.; Li, F.; Lu, J.; Liu, S.; Dorko, K.; Xie, W.; Ma, X. Disposition, Bedaquiline Metabolism: Enzymes and Novel Metabolites. *Drug Metab. Dispos.* **2014**, *42* (5), 863−866, DOI: 10.1124/dmd.113.056119.

(9) Liu, K.; Li, F.; Lu, J.; Liu, S.; Dorko, K.; Xie, W.; Ma, X. Bedaquiline metabolism: enzymes and novel metabolites. *Drug Metab. Dispos.* **2014**, *42* (5), 863−866.

(10) US Food and Drug Administration Center for Drug Evaluation and Research. Application Number 204384Orig1s000, Clinical Pharmacology and Biopharmaceutics review(s) 2012. http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/204384Orig1s000MedR_.pdf.

(11) Natalie, M.; Margino, S.; Erik, H.; Verheyen, G. R.; Van Goethem, F.; Van Gompel, J. Screening for phospholipidosis induced by central nervous drugs: Comparing the predictivity of an in vitro assay to high throughput in silico assays. *Toxicol. In Vitro* **2010**, *24* (5), 1417−1425.

(12) Hyrsova, L.; Vanduchova, A.; Dusek, J.; Smutny, T.; Carazo, A.; Maresova, V.; Trejtnar, F.; Barta, P.; Anzenbacher, P.; Dvorak, Z.; et al. Trans -resveratrol, but not other natural stilbenes occurring in food, carries the risk of drug-food interaction via inhibition of cytochrome P450 enzymes or interaction with xenosensor receptors. *Toxicol. Lett.* **2019**, *300*, 81−91, DOI: 10.1016/j.toxlet.2018.10.028.

(13) Bavaresco, L.; Lucini, L.; Busconi, M.; Flamini, R.; et al. Wine Resveratrol: From the Ground Up. *Nutrients* **2016**, *8*, No. 222, DOI: 10.3390/nu8040222.

(14) Ashrafizadeh, M.; Rafiei, H.; Mohammadinejad, R.; Farkhondeh, T.; Samarghandian, S. J. C. C. I. Anti-tumor activity of resveratrol against gastric cancer: a review of recent advances with an emphasis on molecular pathways. *Cancer Cell Int.* **2021**, *21* (1), No. 66, DOI: 10.1186/s12935-021-01773-7.

(15) Gomes, B. A. Q.; Silva, Q.; Romeiro, C. F. R.; dos Santos, S. M.; et al. Neuroprotective Mechanisms of Resveratrol in Alzheimer's Disease: Role of SIRT1. *Oxid. Med. Cell. Longevity* **2018**, *2018*, No. 8152373, DOI: 10.1155/2018/8152373.

(16) Detampel, P.; Beck, M.; Krahenbuhl, S.; Huwyler, J. Drug interaction potential of resveratrol. *Drug Metab. Rev.* **2012**, *44* (3), 253−265.

(17) Deng, R.; Xu, C.; Chen, X.; Chen, P.; Wang, Y.; Zhou, X.; Jin, J.; Niu, L.; Ying, M.; Huang, M.; Bi, H. Resveratrol suppresses the inducible expression of CYP3A4 through the pregnane X receptor. *J. Pharmacol. Sci.* **2014**, *126* (2), 146−154.

(18) Zhan, Y. Y.; Liang, B. Q.; Li, X. Y.; Gu, E. M.; Dai, D. P.; Cai, J. P.; Hu, G. X. The effect of resveratrol on pharmacokinetics of aripiprazole in vivo and in vitro. *Xenobiotica* **2016**, *46* (5), 439−444.

(19) Meesters, R. J.; Voswinkel, S. Bioanalytical Method Development and Validation: from the USFDA 2001 to the USFDA 2018 Guidance for Industry. *J. Appl. Bioanal.* **2018**, *4* (3), 67−73, DOI: 10.17145/jab.18.010.

(20) Kawamura, K.; Fukumura, S.; Nikaido, K.; Tachi, N.; Kozuka, N.; Seino, T.; Hatakeyama, K.; Mori, M.; Ito, Y. M.; Takami, A.; Hinotsu, S.; Kuno, A.; Kawasaki, Y.; Horio, Y.; Tsutsumi, H. Resveratrol improves motor function in patients with muscular dystrophies: an open-label, single-arm, phase IIa study. *Sci. Rep.* **2020**, *10* (1), No. 20585.

(21) Conradie, F.; Bagdasaryan, T. R.; Borisov, S.; Howell, P.; Mikiashvili, L.; Ngubane, N.; Samoilova, A.; Skornykova, S.; Tudor, E.; Variava, E.; Yablonskiy, P.; Everitt, D.; Wills, G. H.; Sun, E.; Olugbosi, M.; Egizi, E.; Li, M.; Holsta, A.; Timm, J.; Bateson, A.; Crook, A. M.; Fabiane, S. M.; Hunt, R.; McHugh, T. D.; Tweed, C. D.; Foraida, S.; Mendel, C. M.; Spigelman, M. Bedaquiline-Pretomanid-Linezolid Regimens for Drug-Resistant Tuberculosis. *N. Engl. J. Med.* **2022**, *387* (9), 810−823.
(22) Shi, L.; Jiang, Z.; Zhang, B.; Tang, C.; Xu, R. A. Development of UPLC-MS/MS method for studying the pharmacokinetic interactions of pexidartinib with antifungal drugs in rats. *J. Pharm. Biomed. Anal.* **2020**, *188*, No. 113386.
(23) Lan, T.; Fang, P.; Ye, X.; Lan, X.; Xu, R. A. Evaluation of herb-drug interaction of ambrisentan with shikonin based on UPLC-MS/MS. *Pharm. Biol.* **2021**, *59* (1), 1133−1138.
(24) Chow, H. H.; Garland, L. L.; Hsu, C. H.; Vining, D. R.; Chew, W. M.; Miller, J. A.; Perloff, M.; Crowell, J. A.; Alberts, D. S. Resveratrol modulates drug- and carcinogen-metabolizing enzymes in a healthy volunteer study. *Cancer Prev. Res.* **2010**, *3* (9), 1168−1175.
(25) Choi, J. S.; Choi, B. C.; Kang, K. W. Effect of resveratrol on the pharmacokinetics of oral and intravenous nicardipine in rats: possible role of P-glycoprotein inhibition by resveratrol. *J. Pharm. Sci.* **2009**, *64*, 49−52.
(26) Hong, S. P.; Choi, D. H.; Choi, J. S. Effects of resveratrol on the pharmacokinetics of diltiazem and its major metabolite, desacetyldiltiazem, in rats. *Cardiovasc. Ther.* **2008**, *26* (4), 269−275.
(27) Kuhlin, J.; Sturkenboom, M. G. G.; Ghimire, S.; Margineanu, I.; van den Elsen, S. H. J.; Simbar, N.; Akkerman, O. W.; Jongedijk, E. M.; Koster, R. A.; Bruchfeld, J.; Touw, D. J.; Alffenaar, J. C. Mass spectrometry for therapeutic drug monitoring of anti-tuberculosis drugs. *Clin. Mass Spectrom.* **2019**, *14*, 34−45.

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