Research Article

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2021.34.005489

Pharmacokinetic Study of Ethambutol and Amikacin in Rat Lung Tissue Detected by UPLC-MS Method

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ARTICLE INFO

Received: February 05, 2021

Published: February 18, 2021

Citation: Lin Yin, Huichun Shi, Mengting Chen, Jianglei Yu, Ping Jiao, Lijun Zhang. Pharmacokinetic Study of Ethambutol and Amikacin in Rat Lung Tissue Detected by UPLC-MS Method. Biomed J Sci & Tech Res 34(1)-2021. BJSTR. MS.ID.005489.

Abbreviations: TB: Tuberculosis; EMB: Ethambutol; AMK: Amikacin; TCA: Trichloroacetic Acid; MDR : Multidrug-Resistant; XDR: Extensively Drug-Resistant; TDM: Therapeutic Drug Monitoring; INH: Isoniazid; FQ: Fluoroquinolone; CAP: Capreomycin, KAN: Kanamycin; CLA: Clofazimine, MOX: Moxifloxacin; LFX: Levofloxacin; LZD: Linezolid; USP: United States Pharmacopeia; QC: Quality Control

ABSTRACT

Lower drug concentrations at tuberculosis (TB) infection sites may result in drug resistance. Understanding the tissue penetration of anti-TB drugs will offer new clues for optimizing drug dose. In this work, we performed pharmacokinetic study of ethambutol (EMB) and amikacin (AMK) in rat lung. Herein, a total of 160 rats were randomized to 16 time points, including before dosing (0h), and after dosing 0.25, 0.33, 0.5, 0.67, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 8.0, 9.0, 12.0, 16.0 and 24.0h. The drug doses were 90mg/kg intragastric administration for EMB, and 0.20mg/kg intramuscular injection for AMK. The lung homogenate was precipitated with 15% trichloroacetic acid (TCA), diluted with 20% acetonitrile with 0.1% formic acid and 2% heptafluorobutyric acid. The compounds were detected by UPLC-MS/MS. The linear range detected by UPLC-MS/MS was from 10 to 5000 ng/mL ($r^2 \geq 0.99$) for both drugs. The maximum tissue concentrations (C_{max}) were 39890 and 13855ng/mL at 2.25h for EMB and 0.40h for AMK, respectively. The terminal elimination half-life ($t1/2_z$) was approximately of 3.43h and 14.56h, respectively. Their estimated tissue penetration ratios were 107% for EMB and 0.1% for AMK. In conclusion, in this work, a simple UPLC-MS method was developed to perform PK study of EMB and AMK in rat model. Although it is animal PK study, our work might provide a reference for the lung lesion concentration in TB patients. It is necessary to pay attention to AMK usage in severe pulmonary tuberculosis due to its low penetration.

Keywords: Pharmacokinetics; UPLC-MS/MS; Tuberculosis; Lung; EMB; AMK

Introduction

Despite previous successes in management of tuberculosis (TB), the global emergence of multidrug-resistant TB (MDR-TB) and extensively drug-resistant (XDR)-TB has become highly problematic and has led to high mortality [1]. Poor outcome may be due to poor treatment adherence, lack of drugs and lower drug exposure, especially at the site of disease [2-6]. Therapeutic drug monitoring (TDM) of TB drugs has been used to optimize clinical treatment by measuring systemic drug levels in blood/plasma as a guide for individual dose adjustments [2,7-9]. In MDR and XDR-TB treatment, first-line drugs such as EMB and isoniazid (INH) and second-line drugs are usually combined [10]. These second line drugs include several fluoroquinolone (FQ) compounds as well as the injectable drugs AMK, capreomycin (CAP), and kanamycin (KAN) [11]. According to previous reports, the concentration of some drugs in plasma is lower than the recommend ranges [8,11], and much lower in disease tissues including lung. Recently, researchers have tested drug concentrations in lung tissue [12,13], and lesion sites [13], as well as the dialysate [12-14] in the pulmonary cavity, to understand the drug penetration of lung tissue. It was found that clofazimine (CLA), moxifloxacin (MOX) and levofloxacin (LFX) has higher tissue penetration, RIF, PZA and linezolid (LZD) with median, and INH and KAN lower [12,13]. However, so far, the knowledge of the penetration of AMK and EMB in lung tissues was very limited. In this work, we developed an UPLC-MS/MS method to quantify AMK and EMB concentration in lung tissues, and carried out pharmacokinetic study in rat model.

Materials and Methods

Chemicals and Reagents

EMB was purchased from United States Pharmacopeia (USP) with purity of 100%, EMB-d4 (the internal standard (IS) of EMB) from Toronto Research Chemicals with purify of 98% in chemical, 99.3% in isotopic and 97.49% in d4 component. AMK was from China Institute for the Control of Pharmaceutical and Biological Products with purity of ≥ 74.91%, Apramycin sulfate (APM; the IS of AMK) from Dr. Ehrenstorfer, with the purity of 89.6%. HPLC grade methanol and acetonitrile were brought from Merck (Merck, Darmstadt, Germany). Ultra-pure water was made using the Milli-Q system (Millipore, Bedford, MA, USA). Other regents and solvents were chromatographically pure and from Guoyao Company.

UPLC-MS/MS Conditions

The UPLC-MS/MS system consisted of a Waters Acquity UPLC (Waters Corporation, Milford, USA) and an AB Sciex Triple Quad 5500 (AB SCIEX company, Boston, USA). The chromatographic separation of EMB and AMK, and their ISs were conducted on an ACQUITY UPLC HSS T3 column (2.1mm x 100mm, 1.8μm). A 5-μL sample was loaded and eluted with a gradient of water containing 0.1% FA (A) and ACN containing 0.1% FA (B), at 0.35mL/min flow rate with the flowing program: 0–1.5min, 2-80% B; 1.5–2.0min, 80%; 2.0–2.01min, 80-2%; 2.01–3.0min, 2%. The analytes were detected by multiple reaction monitoring (MRM) mode.

Preparation of Standard Curve and Quality Control Solution

The stock solutions of EMB and AMK, at a concentration of 1.0mg/mL, were prepared using 10% methanol, and stored at -80 °C. The working solutions containing EMB and AMK were prepared by diluting the mixed stock solution with 20% methanol to obtain different concentrations (25,000, 22,500, 5000, 1000, 500, 100, 50ng/mL). The IS working solutions were prepared with 20% methanol to obtain the concentration of 0.25μg/mL for EMB-d4, and 5μg/mL for APM. All working solutions were stored at 4 °C. The standard curves and the quality control (QC) samples containing EMB and AMK, were obtained by mixing the corresponding working solutions (40μL), IS (40μL) with 200μL of blank tissue homogenate. The final concentrations were 5000, 4500, 1000, 200, 100, 20, 10ng/mL for the standard curve, and 10, 40, 400, and 4000ng/mL for LLOQ, LQC, MQC and HQC.

Sample Preparation

Tissue homogenate (200μL), 40μL IS and 40μL 20% methanol were mixed, and protein precipitated by 480μL trichloroacetic acid (TCA) at the concentration of 15%. The mixture was vortexed for 3min and centrifuged at 12,000rpm at 4 °C for 10min. Then the supernatant (100μL) was diluted with 100μL solution (20% ACN, 0.1% FA and 2% HFBA, and transferred to an auto-sampler for analysis by UHPLC-MS/MS. The injected volume was 5μL.

Method Validation

Method validation was performed according to Chinese pharmacopoeia 2015, and FDA guidelines [15]. The method was validated for selectivity, carryover, linearity, accuracy, precision, matrix effect, recovery, and stability. The details were shown in the supplement materials.

Pharmacokinetic study in Rat

All animal experiments were performed in accordance with the Animal Care and Use Guideline, and authorized by the Animal Care and Use of Committee of Shanghai Public Health Clinical Center (2017-S017-02). Sprague-Dawley rats (n = 160, equal mix of male and female, weight 180 ± 20g) were obtained from Shanghai Public Health Clinical Center (Shanghai, China). The animals were housed in an air-conditioned room with temperature of 23–26 °C, relative humidity of 40%–60%, and light of 12h on and 12h off. Food and water were freely available. Rats were randomly assigned to 16 time points, including before dosing (0h), and after dosing 0.25, 0.33, 0.5, 0.67, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 8.0, 9.0, 12.0, 16.0 and 24.0h. After a 7-day adaption, all animals were fasted overnight, and used for experiments. EMB was administered at 90mg/kg [intragastric administration (i.g.)] and AMK at 20mg/kg [intramuscular injection (i.m.)]. The rats were euthanized at different time points. The lung tissues were collected, homogenized in PBS (weight to volume of 1:5, w:v), and centrifuged at 12,000rpm for 10min. The supernatants were collected and stored at -80 °C until further analysis.

Data Analysis

All data were expressed as mean ± standard deviation (SD). The PK analysis was performed in DAS 2.0 software, and PK parameters from non-compartment model were collected, including Cmax (the maximal drug plasma concentration); T_{max} (the time to reach C_{max}); AUC_{0-t} (the area under the plasma concentration–time curve from 0 to the time t); $AUC_{0-\infty}$ (the area under the plasma concentration–time curve from 0 to infinity); $t_{1/2}$ (the elimination half-life); CL (clearance), and Vd (apparent volume of distribution).

Incurred Sample Reanalysis

An incurred sample reanalysis (ISR) was carried out to evaluate the reproducibility of the analytical method. Two samples from each time point were randomly selected, except that before dosing, after dosing at 4.0, 12.0, and 24.0h. Not enough volume was left for the samples at 4.0 and 12.0h time points. The receipt standard for ISR was a percentage change less than 20% in 67% samples.

Supplement Methods

Selectivity and Carryover: To investigate the selectivity of the method, six different sources of blank rat lung tissue homogenate were used to make blank, blank spiked with EMB (IS, EMB-d4) and AMK (IS, APM) at the concentration of LLOQ. In addition, lung tissues from a rat after an administration of EMB and AMK for 3 h

were prepared. The presence of interfering peaks in the retention times of the analytes and their ISs was evaluated. Carryover was checked by analyzing a blank matrix sample following HQC.

Linearity: Seven standard curve samples with a series of concentrations ranging from 10-5000ng/mL were prepared as described in "Preparation of standard curve and quality control solution" The analyte-to-IS peak ratios were plotted against the corresponding EMA or AMK concentrations.

Intraday and Interday Precision and Accuracy: Six replicates of LLOQ, LQC, MQC and HQC samples were prepared and analyzed on the same batch and on different batches to determine the intraday and interday precisions and accuracy.

Recovery and Matrix Effect: Extraction recovery was determined by comparing the peak areas of the analyte and IS obtained from the QC samples with those from post-extraction spiked samples (n=6). The matrix effect was calculated by comparing the mean peak areas of QC standards added into post-extracted blank matrix samples with that of pure standard solution at the same concentration.

Stability: Two kinds of QC samples (HQC and LQC; n = 6) were used to evaluate the stability of EMB and AMK in rat lung homogenate under different storage conditions including:

1) Storage at room temperature, or 4 °C for 24h before extraction,

2) Storage at room temperature or 4 °C for 24h after extraction,

3) The stability of freeze-thaw performed by storing at -80 °C and thawing at room temperature every 24h for three cycles,

4) Storage at -80 °C for 30 days.

Results

UPLC-MS Method

Method Development: After optimizing the mass spectrometry parameters, EMB, EMB-d4, AMK, and APM were detected to have suitable MRM ion pairs of m/z 205.1/116.1 for EMB, 209.1/120.2 for EMB-d4, 586.3/425.3 for AMK, and 540.1/217.1 for APM. For chromatograph condition, since EMB and AMK have strong hydrophilicity, we investigated different protein precipitation methods, and dilution solutions at the last step for sample treatment. Our results showed that 15% TCA gave good extraction recovery, and 20% ACN with 0.1% FA and 2% HFBA improved the chromatographic retention capacity of AMK. Representative chromatograms were shown in (Figure 1). Our results showed that there was no significant interference observed from rat lung homogenate at the retention time of EMB, AMK, and their ISs (EMB-d4 and APM). Furthermore, no carryover from residues on the column was observed from the blank lung homogenate after an HQC injection.

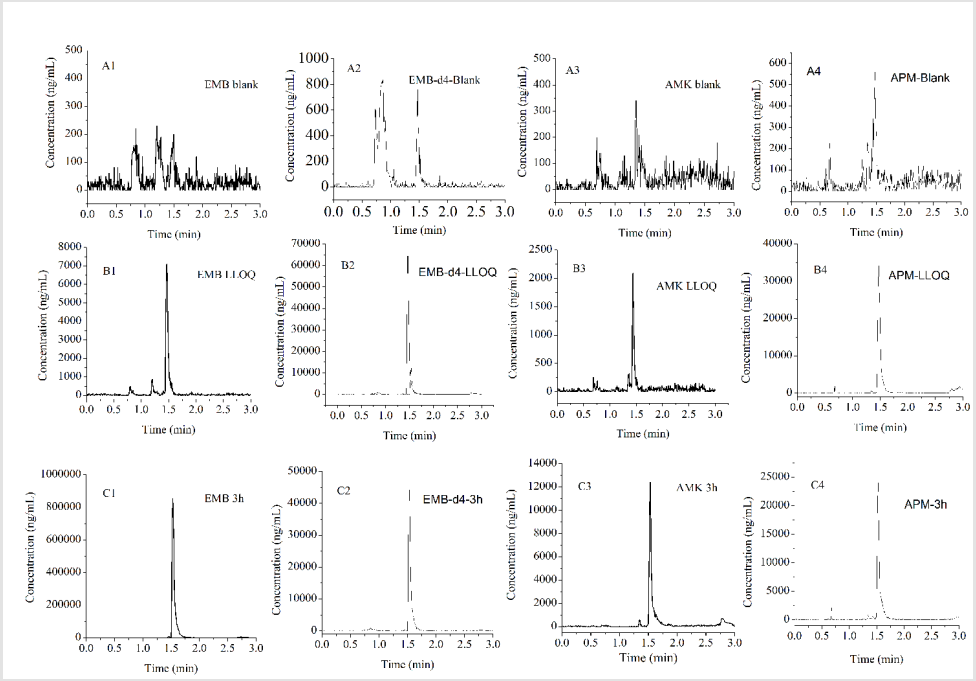

Figure 1: The representative MRM chromatograms of EMB, AMK, and their ISs.

A. EMB (A1), EMB-d4 (A2), AMK (A3) and APM (A4) in blank rat lung homogenate loaded after HQC;

B. Blank rat lung homogenate spiked with EMB (B1), and AMK (B3) at LLOQ, and their ISs (EMB-d4 (B2)and APM (B4));

C. Rat lung homogenate of EMB (C1), and AMK (C3) at 3 h after i.g. EMB and i.m. AMK, and spiked with EMB-d4 (C2) and APM (C4).

Method Validation: The approach provided good linearity in the range of 10-5000ng/mL for both EMB and AMK in rat lung homogenate. The lower limit of quantification (LLOQ) was both 10ng/mL. The intraday accuracy ranged from -5.52% to 1.8% for EMB and -6.6% to 7.08% for AMK, respectively. The interday accuracy ranged from 0.43% to 3.36% for EMB and -2.57% to 5% for AMK, respectively. The intraday and interday precision of EMB and AMK were all less than 11.14%. These data indicated that the method had good precision and accuracy (Table 1). The mean recovery rate of IS normalized EMB and AMK ranged from 98.62% to 106.12% and 97.01%–104.22%, respectively. The matrix effects were from 96.33%–104.14% for EMB, and 94.23%–104.38% for AMK (Table 2). The pre- and post-preparative, freeze-thaw, and long-term stability were measured by comparing six replicates of two QC samples (low and high levels) of EMB and AMK. As shown in (Table 3), the stability results (RSD% and RE%) were all less than 15% in rat lung homogenate under six storage conditions: room temperature or 4 ℃ for 24h pre- or post- preparation, freeze-thaw, and -80 ℃ for 30 days.

Table 1: Intraday and interday precision, the accuracy of EMB and AMK in rat lung (n = 6).

Drug Name	Spiked Concentration (ng/mL)	Intraday			Interday		
		Measured (ng/mL)	Precision(%)	Accuracy(%)	Measured (ng/mL)	Precision(%)	Accuracy(%)
EMB	10	9.45 ± 0.63	6.71	-5.52	10.34 ± 1.14	11.06	3.36
	40	40.35 ± 1.47	3.65	0.90	40.92 ± 2.80	6.84	2.30
	400	396.4 ± 4.77	1.20	-0.90	401.73 ± 29.09	7.24	0.43
	4000	4072 ± 85.56	2.10	1.80	4090.67 ± 204.78	5.01	2.27
AMK	10	10.71 ± 0.88	8.18	7.08	10.35 ± 1.05	10.10	3.49
	40	37.36 ± 4.07	10.89	-6.60	38.97 ± 4.34	11.14	-2.57
	400	391.6 ± 22.51	5.75	-2.10	406.8 ± 25.75	6.33	1.70
	4000	4064 ± 259.77	6.39	1.60	4200 ± 188.53	4.49	5.00

Table 2: Extraction recoveries and matrix effects of EMB and AMK after IS normalized (n=6).

Spiked Concentration (ng/mL)	Matrix Effect (%)		Extraction Recovery (%)	
	EMB	AMK	EMB	AMK
40	104.14	96.85	98.62	97.01
400	103.78	104.38	100.34	102.01
4000	96.33	94.23	106.12	104.22

Table 3: Stability of EMB and AMK in rat lung homogenate under different storage conditions (n=6).

Compounds	Storage conditions	Concentration(ng/mL)		RSD (%)	RE (%)
		Spiked	Measured		
EMB	Room temperature for 24h pre-preparation	40	44.47	2.5	11.18
		4000	4195.67	2.33	4.89
	4 ℃ for 24h pre-preparation	40	45.51	6.81	13.78
		4000	3999.17	11.15	-0.02
	Room temperature for 24h post-preparation	40	45.04	4.72	12.60
		4000	4115.97	5.52	2.90
	Autosampler (4 ℃) for 24h	40	44.38	4.37	10.94
		4000	4131.67	2.5	3.29
	Three-freeze-thaw cycles	40	42.08	1.71	5.21
		4000	4038.89	3.91	0.97
	Long term (-80 ℃) for 30 days	40	43.25	8.78	8.12
		4000	4080.4	3.45	2.01

AMK	Room temperature for 24h pre-preparation	40	44.53	10.4	11.33
		4000	4261.25	0.88	6.53
	4 °C for 24h pre-preparation	40	40.91	13.96	2.27
		4000	3992.50	11.65	-0.19
	Room temperature for 24h post-preparation	40	42.11	13.14	5.28
		4000	4015.00	9.69	0.38
	Autosampler (4 °C) for 24h	40	44.66	10.26	11.64
		4000	3906.67	3.54	-2.33
	Three-freeze-thaw cycles	40	41.52	4.65	3.80
		4000	4045.83	3.09	1.15
	Long term (-80 °C) for 30 days	40	39.16	9.10	-2.10
		4000	4053.6	4.20	1.34

Pharmacokinetic Results

The concentration of EMB (i.g.) and AMK (i.m.) in lung homogenate from 16 time points (n = 10) was determined using the newly developed UPLC-MS/MS approach. Statistical analysis of PK was performed by DAS 2.0 software (Tables S1 & S2). The pharmacokinetic parameters of EMB and AMK using the non-compartmental model were listed in (Table 4). Mean concentration-time curves of EMB and AMK in lung tissue were shown in (Figure 2). EMB and AMK reached a Cmax of 39890 and 13855ng/mL at 2.25 an 0.40h, with $t1/2_z$ of 3.43 and 14.56 h, V_z/F of 3.07 and 0.014 L/kg, respectively. Their estimated doses (using AUC*V*k) in lung tissue are 97.6mg/kg (about 107% of i.g. dose (90mg/kg)) and 0.20mg/kg (only about 0.1% of i.m. dose (20mg/kg)) for EMB and AMK, respectively. In order to detect the accuracy of analysis results, we performed ISR of 24 samples. As shown in (Table 5), the differences between ISR and initial concentration were less than 20% of their mean value except 2 samples including one 30min time sample with 30.3% for EMB, and one 20min time sample with -21.7% for AMK.

Table S1: The detected concentration of EMB in the lung homogenate of pharmacokinetic rat model.

t	No1	No2	No3	No4	No5	No6	No7	No8	No9	No10	Mean	SD	RSD/%
0	0	0	0	0	0	0	0	0	0	0	0		
0.25	153	353	746	166	75	127	526	501	969	555	417.1	296.045	70.977
0.33	303	482	1030	264	237	560	600	100	1200	1300	607.6	425.926	70.1
0.5	2410	4160	1670	960	1900	716	827	4260	1500	1800	2020.3	1267.204	62.724
0.67	4050	5330	3380	3540	4410	1150	5000	10000	5000	10000	5186	2800.636	54.004
1	19300	19300	14500	21200	19400	4700	6700	18600	9860	24800	15836	6653.659	42.016
1.5	17900	20900	38100	34400	27700	5860	17600	21800	23400	14900	22256	9399.138	42.232
2	16700	22500	29300	26900	28600	12900	18100	15300	10800	16000	19710	6675.902	33.871
2.5	16500	23500	25700	20200	26500	23000	22300	12500	9580	14200	19398	5853.891	30.178
3	13300	30900	18900	17900	17300	8900	17300	15300	8900	21700	17040	6391.696	37.51
4	8700	12300	15800	15100	19700	8170	15100	15000	4470	13600	12794	4479.031	35.009
8	5250	8730	6090	11600	5780	3390	8600	12500	3350	2900	6819	3418.559	50.133
9	4200	8540	6320	11600	4500	5420	6100	10300	300	1450	5873	3578.821	60.937
12	2790	3830	1870	4050	2550	971	702	844	779	614	1900	1328.605	69.927
16	788	1280	999	1620	188	466	365	2010	230	386	833.2	629.572	75.561
24	467	478	438	351	350	674	372	188	436	371	412.5	123.961	30.051

Table S2: The detected concentration of AMK in the lung homogenate of pharmacokinetic rat model.

t	No1	No2	No3	No4	No5	No6	No7	No8	No9	No10	Mean	SD	RSD/%
0	0	0	0	0	0	0	0	0	0	0			
0.25	8550	11900	8750	8630	8560	6480	7950	5200	7220	5130	7837	1986.566	25.349
0.33	8520	9950	11200	10400	7120	8480	9950	11200	10400	7120	9434	1530.833	16.227
0.5	10300	11800	8120	19500	8740	7850	6320	10600	13200	10200	10663	3698.516	34.686
0.67	5870	8830	8680	6840	8080	6360	5000	8000	9000	8800	7546	1426.287	18.901

1	6660	4560	4860	6360	7200	4220	4970	4660	3880	5920	5329	1125.546	21.121
1.5	2180	3070	3160	3780	3330	1560	1950	2110	2890	2650	2668	700.853	26.269
2	1360	2250	2500	1730	1990	1580	1550	1090	1070	1710	1683	465.022	27.631
2.5	1230	2060	1940	1790	3090	1170	1340	1760	1710	957	1704.7	606.843	35.598
3	2040	2580	2400	1670	1250	1050	1100	986	1170	1020	1526.6	607.209	39.775
4	643	537	639	449	609	904	544	591	590	660	616.6	118.696	19.25
8	451	629	512	695	579	431	135	864	462	505	526.3	191.046	36.3
9	651	499	425	555	2210	424	868	613	543	417	720.5	540.787	75.057
12	442	370	295	384	322	324	345	337	492	397	370.8	60.319	16.267
16	300	372	349	393	309	322	132	133	133	134	257.7	110.787	42.991
24	436	394	372	267	382	715	395	329	564	418	427.2	126.597	29.634

Table 4: Main pharmacokinetic parameters of EMB and AMK in rat lung after i.g. and i.m. administration.

Parameters	Unit	i.g. EMB	i.m. AMK
Dose	mg/kg	90	20
$AUC_{(0-t)}$	μg/L.h	155,715.03	23,011.06
$AUC_{(0-\infty)}$	μg/L.h	157,280.69	29,252.39
$CL_{z/F}$	L/h/kg	0.62	0.001
$V_{z/F}$	L/kg	3.07	0.014
$MRT_{(0-t)}$	h	5.06	4.78
$MRT_{(0-\infty)}$	h	5.32	14.47
C_{max}	μg/L	39,890	13,855
$t1/2_z$	h	3.43	14.56
T_{max}	h	2.25	0.40

Table 5: Incurred sample reanalysis of EMB and AMK.

Sampling point	EMB				AMK			
	Initial conc. (ng/mL)	Re-assay conc. (ng/mL)	Mean	Difference (%)	Initial conc. (ng/mL)	Re-assay conc. (ng/mL)	Mean	Difference (%)
15min-2	80.4	70.5	75.5	13.1	3110	3160	3135	-1.6
15min-3	172	149	160.5	14.3	1930	2310	2120	-17.9
20min-1	67.9	60.6	64.3	11.4	1810	2250	2030	-21.7
20min-3	243	206	224.5	16.5	3400	2960	3180	13.8
30min-1	986	883	934.5	11.0	2250	2730	2490	-19.3
30min-7	224	165	194.5	30.3	1520	1660	1590	-8.8
40min-4	818	708	763	14.4	1520	1800	1660	-16.9
40min-6	257	231	244	10.7	1530	1670	1600	-8.8
1h-5	4440	3880	4160	13.5	1810	1900	1855	-4.9
1h-10	6010	4960	5485	19.1	1550	1560	1555	-0.6
1.5h-3	9240	7620	8430	19.2	678	820	749	-19.0
1.5h-8	5080	4370	4725	15.0	536	541	538.5	-0.9
2h-2	5220	4500	4860	14.8	546	577	561.5	-5.5
2h-5	6620	5720	6170	14.6	453	508	480.5	-11.4
2.5h-2	5430	4700	5065	14.4	560	528	544	5.9
2.5h-10	3210	2840	3025	12.2	196	233	214.5	-17.2
3h-6	5410	4600	5005	16.2	235	257	246	-8.9
3h-9	5310	4380	4845	19.2	306	291	298.5	5.0
6h-1	2480	2240	2360	10.2	131	151	141	-14.2
6h-8	2330	2050	2190	12.8	164	141	152.5	15.1

8h-3	1440	1220	1330	16.5	110	114	112	-3.6
8h-5	1390	1160	1275	18.0	139	132	135.5	5.2
16h-1	190	158	174	18.4	59.2	57.7	58.45	2.6
16h-3	226	200	213	12.2	60.7	70.8	65.75	-15.4

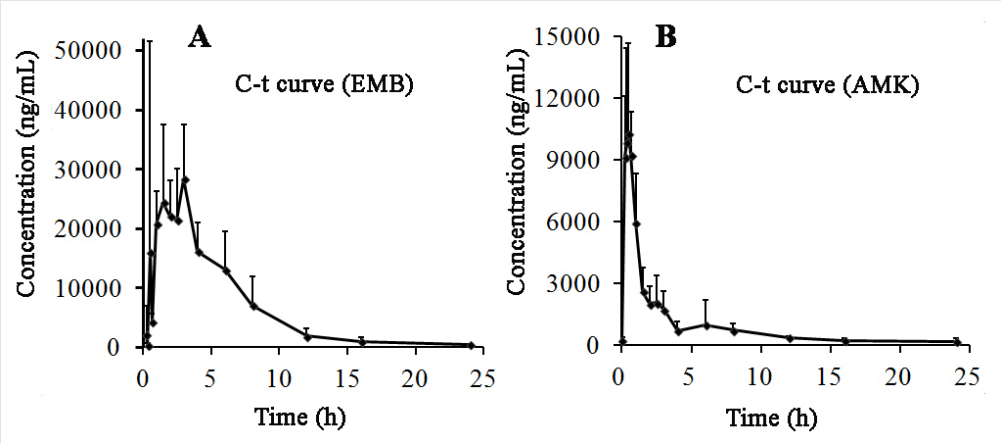

Figure 2: Mean plasma concentration-time curves of EMB (A) and AMK (B) after drug administration for 24h (n=10).

Discussions

Although there are methods reporting quantification of EMB [2,16-20] and AMK [17,21,22] in plasma [2,16-22], milk [21], muscle [21] and wastewater [23] singly, as well as simultaneously in plasma [24] and sputum [25] using LC-MS, no study has reported simultaneous quantification of EMB and AMK in lung tissues. In this work, we developed and validated a simple UPLC-MS/MS method, in which 15% TCA was used for protein precipitation. TCA was chosen as it is dependent on the trichloro-group, and has higher extraction recovery for hydrophilic compounds (such as AMK) [17,21,26]. Furthermore, to improve the retention time of AMK (an aminoglycoside compound) in C18 column, an ion-pairing agent of HFBA was added. HFBA exhibited better retention of aminoglycosides on the analytical column and was also better at enhancing the electrospray process than other ion pair reagents [27,28]. The verified UPLC-MS/MS method was then used for quantitation in a PK study of EMB (i.p.) and AMK (i.m.). Our results showed that EMB had very strong lung penetration (107%), however, AMK was very weak (0.1%). The C_{max} of AMK in lung detected in this work was similar to that previously reported [29,30]. Our results indicate that the developed method was available to detect the concentration of EMB and AMK in the lung tissues from MDR-TB patients.

Conclusion

In this work, a simple UPLC-MS method was developed to perform PK study of EMB and AMK in rat model. Although it is animal PK study, our work might provide a reference for the lung lesion concentration in TB patients. Our study showed that the lung penetration of AMK was very weak. It is necessary to pay attention to AMK usage in severe pulmonary tuberculosis due to its low penetration.

Acknowledgment

We would like to express our gratitude to the grant from National Science and Technology Program during the Thirteenth Five-year Plan Period, China (2017ZX09304027), Shanghai Municipal Commission of Health and Family planning (201640005), and the National Natural Science Funds, China (81271834). We would like to thank Editage (www.editage.com) for English language editing.

Declarations

Not applicable

Conflicts of Interest/Competing Interests

All authors declared no potential conflicts.

Authors' Contributions

a. Lijun Zhang: Conceptualization, Writing, Methodology, Formal analysis, Software, Investigation, Project administration, Funding acquisition.

b. Yin Lin: Resources, Investigation, Data Curation, Validation.

c. Huichun Shi: Writing, Investigation, Data Curation.

d. Chen Mengting: Data curation, Investigation, Methodology.

e. Yu Jianglei: Visualization, Investigation.

f. Ping Jiao: Data curation.

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ISSN: 2574-1241

DOI: 10.26717/BJSTR.2021.34.005489

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