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Determination and pharmacokinetic analysis of ticarcillin disodium–clavulanate potassium for injection in rat plasma by UPLC-ESI-MS/MS

Moli Wang[1,2], Yanxia Gao[2], Xueli Liu[2], Jing Zhang[2], Qiang Wang[2], Junshan Chang[2] and Lantong Zhang[1]

Abstract

Objective: To establish a specific and rapid ultra-high-performance liquid chromatography–electrospray ionization–tandem mass spectrometry (UPLC-ESI-MS/MS) method for measuring ticarcillin and clavulanate levels in rat plasma.

Methods: A Waters ACQUITY BEH C18 column (50 mm × 2.1 mm, 1.7 μm) and SCIEX QTRAP® LC-MS/MS System were used. Analyses were conducted to optimize the chromatographic and MS conditions, and the pharmacokinetic parameters of ticarcillin and clavulanate were assessed.

Results: Linear relationships were observed in the ranges of 10 to 10,000 ng/mL for ticarcillin R ($r^2 = 0.9967$) 30 to 10,000 ng/mL for ticarcillin S ($r^2 = 0.9961$), and 30 to 10,000 ng/mL for clavulanate ($r^2 = 0.9981$). The average extraction recoveries of all compounds ranged from 86.9% to 96.4%. The pharmacokinetic parameters of the ticarcillin R and S isomers in rats were distinctive. The ticarcillin R and S isomers and clavulanate were rapidly absorbed *in vivo*. Ticarcillin S and clavulanate had similar elimination rates, whereas that of ticarcillin R was slower.

Conclusion: A UPLC-ESI-MS/MS method was developed and validated for the determination of ticarcillin and clavulanate in rat plasma.

[1]Department of Pharmaceutical of Analysis, School of Pharmacy, Hebei Medical University, Shijiazhuang, China
[2]Department of Chemical Drug Control, Hebei Institute of Drug Control and Research, Shijiazhuang, China

Corresponding author:
Lantong Zhang, Department of Pharmaceutical of Analysis, School of Pharmacy, Hebei Medical University, No. 361 Zhongshan East Road, Shijiazhuang 050017, China.
Email: lantongzhang@yeah.net

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Clavulanate potassium, *in vivo*, pharmacokinetics, ticarcillin, ultra-high-performance liquid chromatography, mass spectrometry, rat

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Introduction

As a novel thieno-carboxyl drug, ticarcillin is a semi-synthetic anti-*Pseudomonas* penicillin. Previous research demonstrated that ticarcillin has weaker antimicrobial effects against gram-positive bacteria than penicillin G and stronger antimicrobial effects against gram-negative bacteria than carbenicillin.[1] Gram-positive and gram-negative bacteria produce β-lactamases, which can destroy penicillins before they generate antibacterial activity. Therefore, the therapeutic effect of penicillins is lessened. Potassium clavulanate is a strong inhibitor of β-lactamase. Hence, potassium clavulanate and ticarcillin sodium have the potential to be combined into a compound preparation with a broader antibacterial spectrum and stronger antimicrobial effects.[2] A recent study suggested that this combination has good efficacy against community-acquired pneumonia (CAP) in elderly patients.[3] Meanwhile, non-fermentative gram-negative bacilli (NFGNB) exhibit little resistance to the compound preparation.[4] In clinical practice, the injectable compound preparation consists of ticarcillin sodium and clavulanate potassium at a ratio of 15:1. Ticarcillin sodium exhibits R and S isomerism in the compound preparation. The two isomers possess different pharmacokinetic parameters *in vivo*. Although we cannot isolate these two isomers, C18 columns can be used to separate the two molecules. In addition, ticarcillin and clavulanate have similar kinetic characteristics. The blood concentration of ticarcillin is higher in patients treated with ticarcillin and clavulanate than in those treated with ticarcillin alone.[5] However, to the best of our knowledge, no method that simultaneously measures ticarcillin and clavulanate levels in rats has been developed. Methods for the determination of ticarcillin sodium have been included in British Pharmacopoeia (2011 Edition) and European Pharmacopoeia (version 7.0).[6,7] However, this drug is not described in Chinese Pharmacopoeia (2015 Edition). Ultra-high-performance liquid chromatography (UPLC)–tandem mass spectrometry (MS/MS) is a powerful tool applied in pharmacokinetic studies. The advantages of this technology include its high-efficiency separation capability, high selectivity, and high sensitivity. The results provide structural information and simultaneously measure the quantities of different components. We aimed to employ the UPLC–MS/MS method to construct a quantitative system for rapidly and accurately determining ticarcillin and clavulanate levels in rat plasma.

Methods

We followed the guiding principles of biological sample testing of the Food and Drug Administration in our analysis.[8] Approval of the study was granted by the Ethic Committee of Hebei Institute of Drug Control and Research (approval number: 2019 1). As an animal study, the requirement for informed consent was waived.

Animals

Male Sprague–Dawley rats weighing 250 ± 10 g were obtained from Shanghai SLAC

Laboratory Animal Company (certificate number 2008001667671, Shanghai, China). All animal facilities complied with the requirements of the Association of International Laboratory Animals Assessment and Certification. Rats were housed at 22 to 24°C and relative humidity of 50 ± 5% under a 12-hour/12-hour light/dark cycle. Rats were fasted for 12 hours before the experiment but granted free access to water.

Liquid phase conditions

We used an ultra-high-pressure LC system from Waters (Milford, MA, USA). A Waters ACQUITY BEH C18 column (50 mm × 2.1 mm, 1.7 μm) was used in the current study, and the column temperature was set at 30°C. The mobile phases were A (0.1% formic acid in water, v/v) and B (0.1% formic acid in acetonitrile, v/v). Gradient elution was performed as follows: 0.0 to 1.00 minutes, 80% to 10% A; 1.00 to 1.20 minutes, 10% A isocratic elution; 1.20 to 1.21 minutes, 10% to 80% A; and 1.21 to 1.50 minutes, 80% A isocratic elution. Pre-equilibration was performed for 1 minute before each injection, and the flow rate was 0.6 mL/minute. The post-column solution was treated without flow and entered into the ion source. A 2-μL sample was used for further analysis. The total analysis time was 1.5 minutes.

MS conditions

We used the QTRAP® LC-MS/MS System (SCIEX, Framingham, MA, USA) in this experiment. The temperature of the electrospray ionization (ESI) source (StepWave™, Waters) and ion source temperature were consistently set at 150°C. The capillary voltage was 2.0 kV, and the offset voltage was 50 V. The desolvation temperature was adjusted to 500°C, and the flow rate of desolvation was 800 L/hour. The flow rate of cone was 150 L/hour. The nebulizer pressure was 7.0 bar. The interface was heated, and the multiple reaction monitoring mode was used. The test compound and tolbutamide (internal standard) were examined in the positive ion mode.

Reference solution and quality control (QC) samples

Ticarcillin (batch number: 130569-200902, content: 84%, Chinese Institute for Food and Drug Control, Beijing, China) and clavulanate (batch number: 130429-201307, content: 95%, Chinese Institute for Food and Drug Control) were diluted with 70% acetonitrile to create control stock solutions (1.0 mg/mL). Acetonitrile was further used to create dilutions of 100, 60, 30, 10, 3, 1, 0.3, and 0.1 μg/mL for each drug. QC samples were also diluted. For ticarcillin, we obtained a series of concentrations as follows: 80, 24, 0.8, and 0.3 μg/mL. For clavulanate, we obtained a series of concentration as follows: 80, 24, and 0.8 μg/mL. Tolbutamide (200.00 ng/mL, batch number: 100500-200801, content: 100%, Chinese Institute for Food and Drug Control) was used as an internal standard solution. Both ticarcillin sodium and clavulanate potassium are sensitive to light and heat. Thus, the use of similar antibiotics as internal controls results in certain difficulties in detection. Tolbutamide is extremely stable, and it has a large response value in the positive ion mode. In addition, the peak time is similar to that of the test substance. Thus, tolbutamide was selected as the internal standard. The aforementioned solutions were stored at 4°C for further study.

Plasma sample preparation

In total, 20 μL of plasma and 80 μL of the internal standard solution (200.00 ng/mL tolbutamide) were added to a 96-well

plate. The mixture was vortexed for 5 minutes to precipitate protein. After centrifugation at 4000 rpm for 20 minutes, 50 μL of the supernatant was taken and mixed with 200 μL of water. The solution was then filtered through a 0.22-μm microporous membrane.

Specific properties

We tested samples including blank plasma, blank control + reference substance, and blank plasma + sample. Based on the test results, interference with the tested components and internal standards by endogenous substances, metabolites, and exogenous substances was evaluated.

Standard curve, lower limit of quantification (LLOQ), and limit of detection (LOD)

A 5-μL standard solution of ticarcillin or clavulanate was added to 45 μL of rat blank plasma. The test solution was similarly prepared. The peak areas of ticarcillin, clavulanate, and tolbutamide were recorded, and the ratio of the peak area of each test component to that of the internal standard was calculated ($A_{\text{test sample}}/A_{\text{internal standard}}$). The concentration of each measured component in plasma (ng/mL) was plotted on the abscissa, and the ratio of the peak area of each measured component to that of the internal standard ($A_{\text{test sample}}/A_{\text{internal standard}}$) was plotted on the ordinate. Weighted least squares ($W = 1/\chi^2$) was used to fit a linear regression and the standard curve. For the reference solution, we diluted the sample to six concentrations (three parallel samples of each concentration). Each sample was tested three times to obtain a reliable result. The LLOQ was the lowest point on the standard curve of each component under test. Six consecutive measurements were required so that the average concentration of LLOQ measured in the QC sample was within ±20% of the indicated concentration. Meanwhile, the relative standard deviation (RSD) should be ≤20%, and the signal-to-noise ratio (S/N) should be ≥10. The stepwise dilution method was used to measure each control solution (S/N = 3), which yielded the lowest LOD for each component.

Precision and accuracy

The standard QC solution was added to blank plasma. Low (800 ng/mL), medium (24,000 ng/mL) and high (80,000 ng/mL) concentrations of the QC sample solution were prepared (six replicates each). Ticarcillin and clavulanate solutions were prepared at the aforementioned concentrations. The method used for standard curve construction as was applied in this experiment. The concentration of the QC sample was calculated using the standard curve constructed in the same batch. Accuracy was represented by the relative error (RE), and precision was represented by the RSD. RE% was calculated as [(measured value-real value)/real value] × 100%. The intra-day precision and daytime precision of the high, medium, and low concentration solutions were required to be less than 15%, and accuracy was limited to ±15%.

Extraction recovery and matrix effects

In the first part, 5 μL of low-, medium-, and high-concentration QC solutions were added to 45 μL of blank plasma (six replicates each). The sample concentration was measured as previously mentioned. The peak areas of the measured component and internal standard substance were calculated. In the second part, 45 μL of blank plasma were prepared as previously mentioned. Subsequently, 5 μL of low-, medium-, and high-concentration QC solutions were added to the treated blank plasma. The peak areas of the measured

component and internal standard substance were calculated accordingly. In the third part, to obtain the same solution as the aforementioned theoretical concentration, low-, medium-, and high-concentration QC solutions were prepared using the mobile phase. The peak area of the measured component and internal standard substance were calculated using the same method. The extraction recovery was calculated as follows: quantity from the first part/quantity from the second part × 100%. Meanwhile, the matrix effect was calculated as follows: quantity from the second part/quantity from the third part × 100%. The extraction recoveries of the low-, medium-, and high-concentration solution should all exceed 50%. In addition, the RSDs of the medium- and high-concentration solutions should be lower than 15%, and that of the low-concentration solution should be lower than 20%.

Stability test

In total, 45 μL of blank plasma was added to the QC standard solution prepared as previously described. The short-term stability of samples was tested after the samples were stored at room temperature for 24 hours. The long-term stability of samples was tested after the samples were stored at −20°C for 30 days. In addition, stability was examined after three freeze–thaw cycles (−20°C to room temperature).

Plasma sample determination

When measuring plasma samples, a standard curve of each component was simultaneously prepared. The quality of low-, medium-, and high-concentration samples was measured simultaneously. The plasma sample concentration and QC sample concentration were calculated according to the standard curve.

Pharmacokinetic tests in rats

Sprague–Dawley rats were treated with an intravenous injection of ticarcillin disodium and clavulanate (D company, origin: China, batch number: 30511301, specification: 1.6 g) at a dose of 144 mg/kg. Six rats were randomly divided into the treatment and control groups. The measurement was performed in each rat and averaged within groups. Blood samples were collected at 0.08, 0.25, 0.5, 0.75, 1, 2, 4, 6, 9, 12, 18, 24, and 48 hours after injection and stored in heparinized plastic centrifuge tubes. The samples were centrifuged at 4000 rpm for 10 minutes. Plasma was collected and stored at −20°C until analysis. The peak area ratio of each test sample was recorded and used to calculate the concentrations of ticarcillin sodium and clavulanate in plasma. The Ln value of each component concentration was the ordinate, and the time was the abscissa. The blood drug log concentration–time curve was plotted. The pharmacokinetic parameters were calculated using Excel software as recommended by the 2015 edition of the Chinese Pharmacopoeia, which is equivalent to 3P97 software. Pharmacokinetic parameters, including the plasma concentration peak (C_{max}) and time to peak concentration (t_{max}), were obtained from the plotted plasma log concentration–time curves. The elimination half-life ($t_{1/2}$) of the drug was calculated as follows: $t_{1/2} = 0.693/k$.

Results

Method optimization

MS conditions

To confirm the MS conditions, we examined different mass spectrum parameters, including the retention time and response value in the positive and negative ion modes. The results illustrated that the

retention times substantially differed among clavulanate (0.38 minutes), ticarcillin (0.63 minutes for the S isomer and 0.73 minutes for the R isomer), and the internal standard (0.90 minutes). The response values of the test compound were higher in the positive ion mode than in the negative ion mode. However, the response values of the internal standard sample were similar in both modes. Based on the aforementioned results, we chose to test the sample in the positive ion mode. Figure 1 presents the reference chromatograms of ticarcillin and clavulanate in blank plasma samples. The parent/daughter ion pairs of the test compound and internal standard were as followed: ticarcillin S, m/z 385.2/204.2; ticarcillin R, m/z 385.2/160.2; clavulanate, m/z 197.9/136.0; and tolbutamide, m/z 271.1/91.1. The mass spectra of ticarcillin and clavulanate in plasma samples are presented in Figure 2.

Method validation

Specificity. Blank plasma, blank plasma + internal standard, and rat plasma samples after intravenous injection were analyzed using the optimized experimental conditions. Figures 3, 4, and 5 suggested

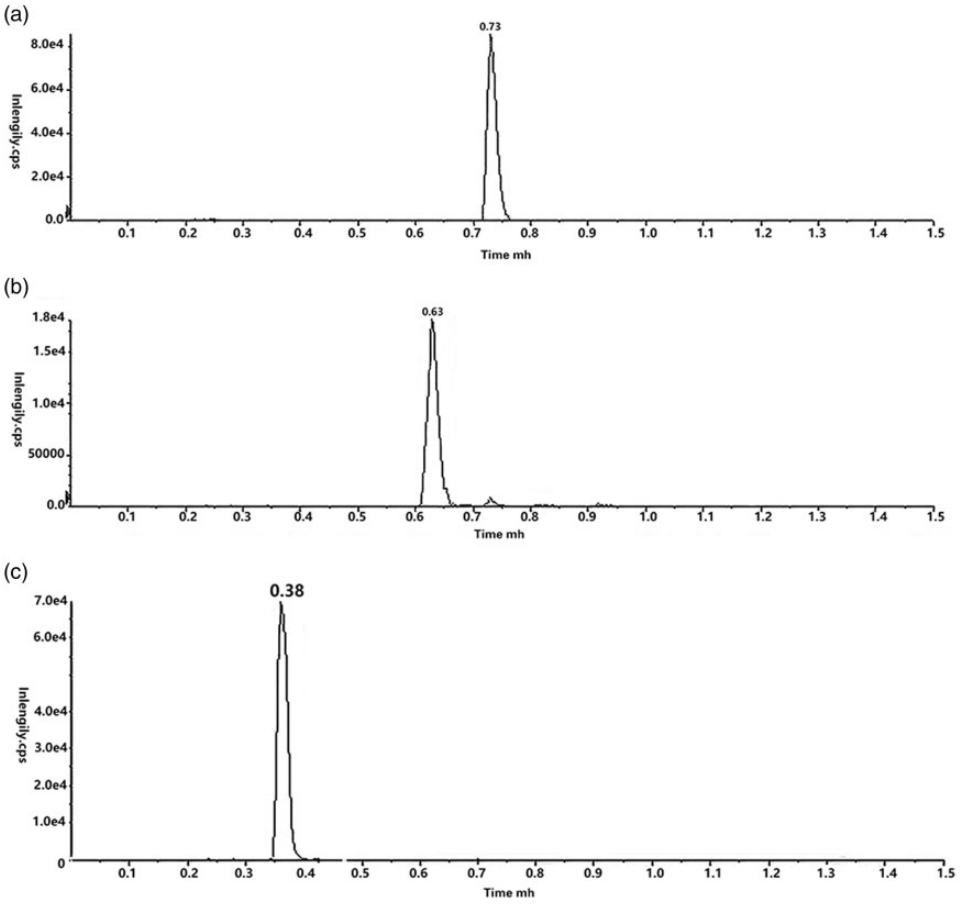

Figure 1. Chromatograms of (a) ticarcillin and (b) clavulanate in blank plasma.

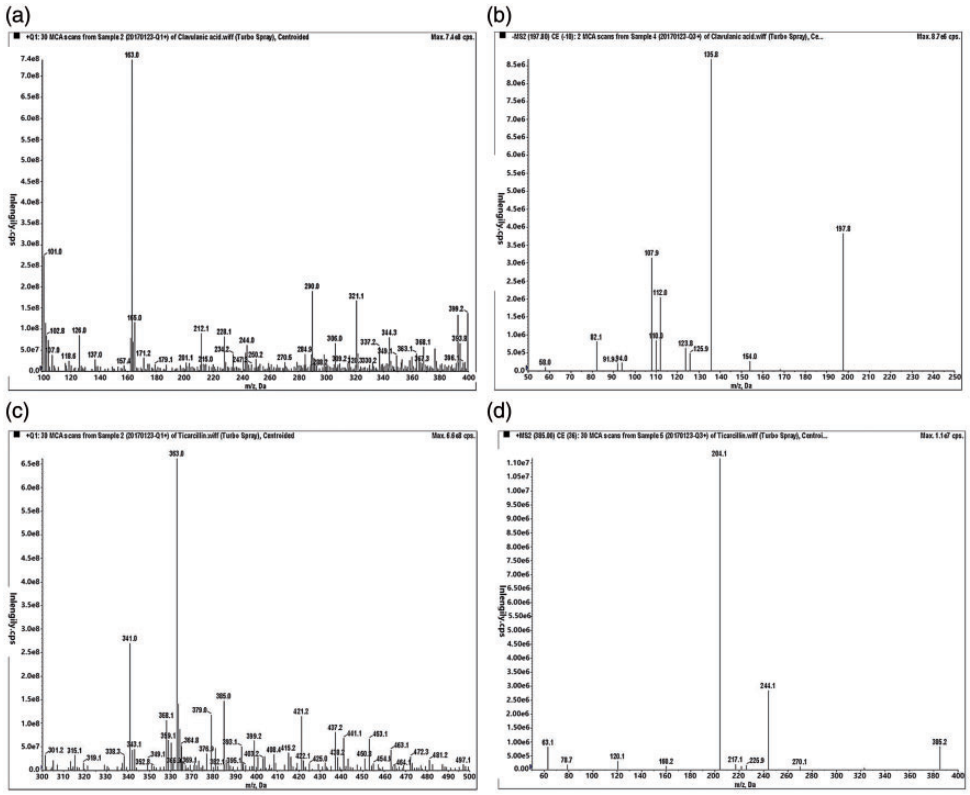

Figure 2. Mass spectrometry chromatograms of plasma samples after the intravenous injection of ticarcillin and clavulanate. (a) Mass spectra of the molecular ion peak of clavulanate in the first-order mass spectrometry scanning mode. (b) Mass spectra of the molecular ion peak of clavulanate in the second-order mass spectrometry scanning mode. (c) Mass spectra of the molecular ion peak of ticarcillin in the first-order mass spectrometry scanning mode. (d) Mass spectra of the molecular ion peak of ticarcillin in the second-order mass spectrometry scanning mode.

that endogenous substances in rat plasma had no influence on the measurement of ticarcillin, clavulanate, and the internal standards. These results indicated that the specificity of this method was high.

Standard curve, LLOQ, and LOD. Table 1 presents the standard curve, LLOQ, and LOD. The results indicated that ticarcillin and clavulanate had good linearity in the linear range of this study.

Precision and accuracy. The RSDs of intraday precision for ticarcillin and clavulanate were lower than 3.3%, and those of the daytime precision of the drugs were lower than 4.3%. In addition, the intraday REs of the accuracy for ticarcillin and clavulanate ranged from −3% to 2.5%, and the daily REs of accuracy for ticarcillin and clavulanate ranged from −3.7% to 5% (Table 2). The results suggested that the precision and accuracy of this method were good.

Extraction recovery and matrix effect. The extraction recoveries of the three concentrations of ticarcillin and clavulanate

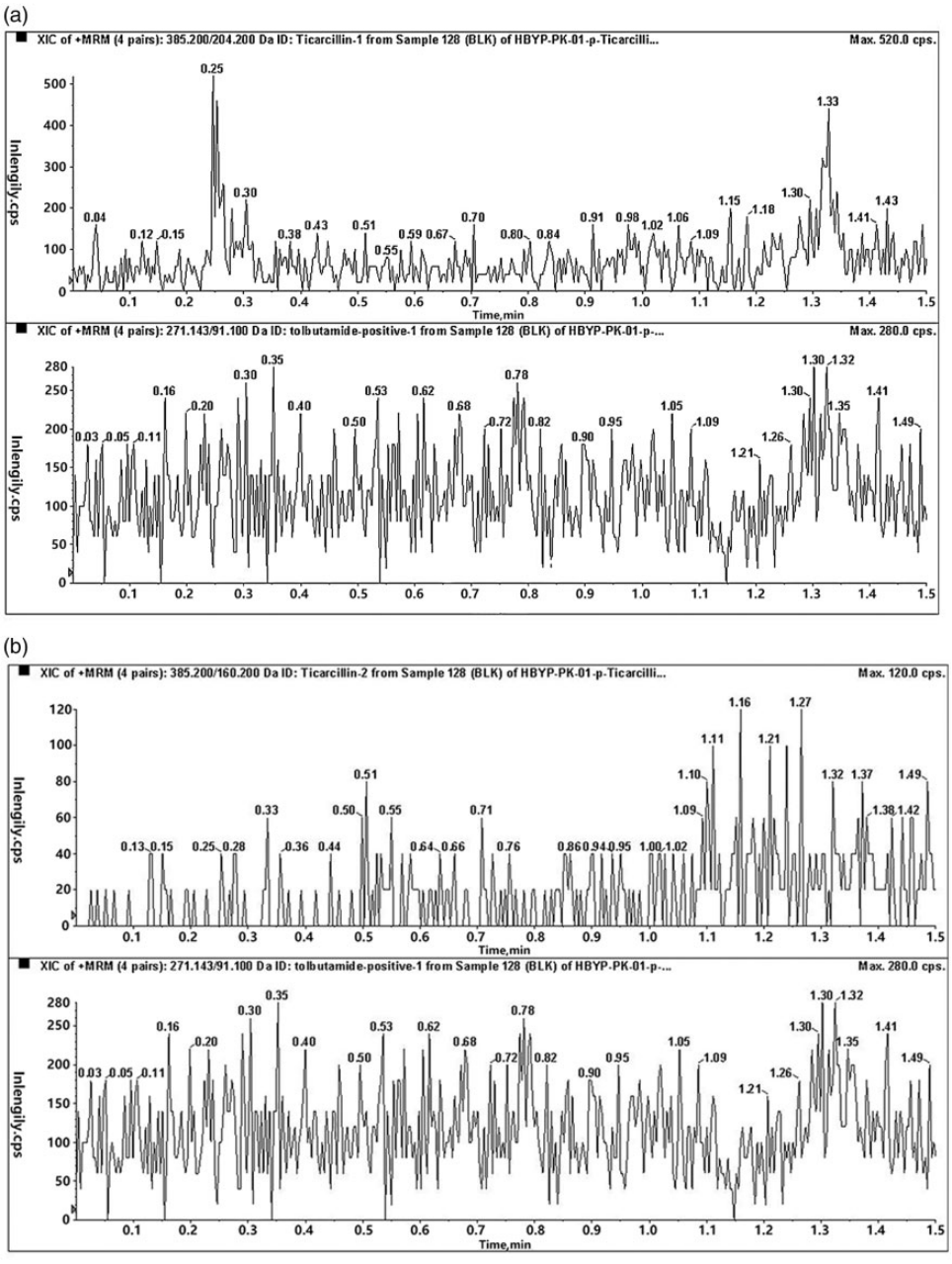

Figure 3. (a, b) Multiple reaction monitoring chromatograms of blank plasma.

(low, medium, and high) ranged from 86.9% to 96.4%. The matrix effect of three concentrations of ticarcillin and clavulanate ranged 97.7% to 101% (Table 3). The results illustrated that endogenous substances in rat plasma had no significant effect on the ionization of the two tested components.

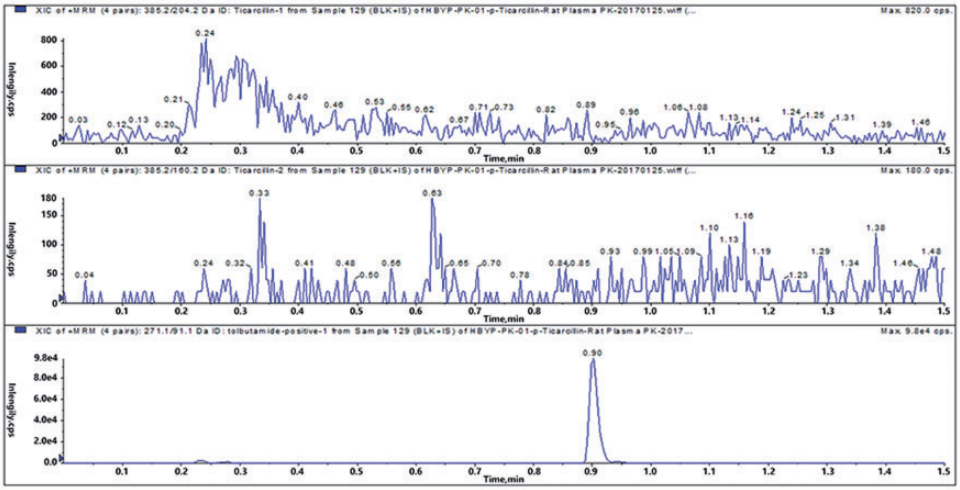

Figure 4. Multiple reaction monitoring chromatograms of tolbulamide (internal standard) in blank plasma.

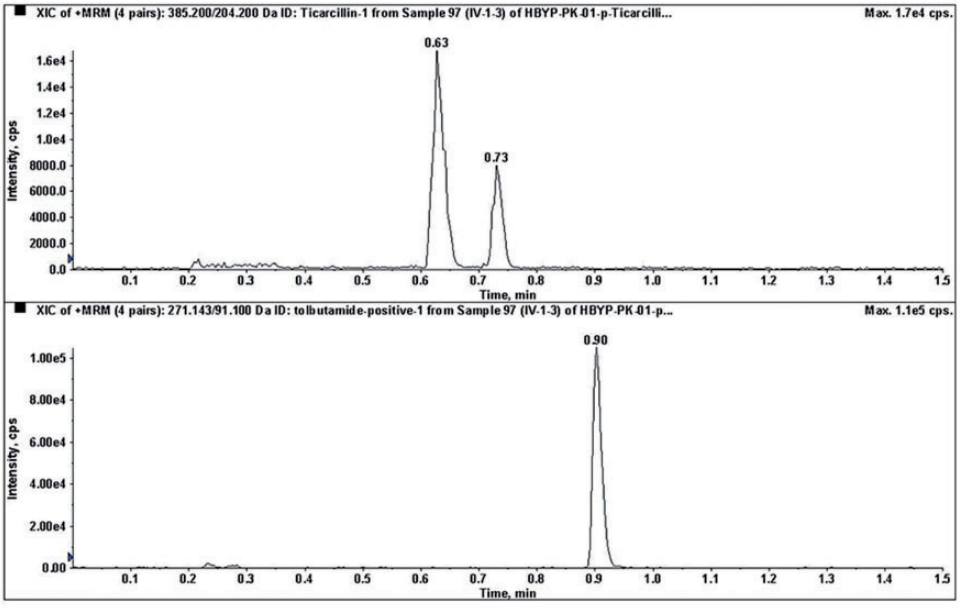

Figure 5. Multiple reaction monitoring chromatograms of ticarcillin, clavulanate and internal standard in plasma samples after the intravenous administration of preparation.

Stability. The short- and long-term stability of ticarcillin and clavulanate are presented in Table 4. The stability values of ticarcillin and clavulanate ranged from −4.3% to 3.2%. Table 5 presents the stability of samples after three freeze–thaw cycles and that of the extracted samples. The accuracy values ranged from −4.7% to 5%, which suggested good stability under the experiment conditions of the proposed method.

Table 1. Standard curves, LLOQs, and LODs of ticarcillin and clavulanate.

Compounds	Regression equation[a]	r^2	Linear range (ng/mL)	LLOQ (ng/mL)	LOD (ng/mL)
Ticarcillin R	Y = 7.96e−5X + 8.67e−4	0.9967	30 to 10,000	10.0	3.5
Ticarcillin S	Y = 3.11e−4X + 4.09e−4	0.9961	10 to 10,000	3.0	1.0
Clavulanate	Y = 5.83e−5X − 5.47e−4	0.9981	30 to 10,000	10.0	3.5

[a]Y: peak area; X: concentration of the compound (ng/mL).
LLOD, lower limit of detection; LOQ, limit of detection.

Table 2. The intraday and interday accuracy and precision of low, medium, and high concentrations of ticarcillin and clavulanate in rat plasma.

		Intraday (n = 6)			Interday (n = 6)		
Compounds	Concentration (ng/mL)	Concentration[a] (ng/mL)	Accuracy (%)	Precision (%)	Concentration[a] (ng/mL)	Accuracy (%)	Precision (%)
Ticarcillin R	30	29.1 ± 0.8	−3.0	2.7	29.8 ± 1.2	−0.7	4.0
	2400	2368 ± 14	−1.3	0.6	2345 ± 14	−2.3	0.6
	8000	8051 ± 93	0.6	1.2	7992 ± 85	−0.1	1.1
Ticarcillin S	30	30.7 ± 0.5	2.3	1.7	28.9 ± 1.3	−3.7	4.3
	2400	2459 ± 31	2.5	1.3	2458 ± 17	2.4	0.7
	8000	8012 ± 86	0.2	1.1	7921 ± 71	−1.0	0.9
Clavulanate	80	78.7 ± 2.0	−1.6	2.5	82.4 ± 1.0	3.0	1.3
	300	294 ± 10	−2.0	3.3	315 ± 11	5.0	3.7
	2400	2355 ± 51	−1.9	2.1	2377 ± 68	−1.0	2.8

[a]Mean ± standard deviation.

Table 3. The mean extraction recoveries and matrix effects of ticarcillin and clavulanate in rat plasma.

	Mean extraction recovery (%)			Matrix effect (%)		
Components	Low	Medium	High	Low	Medium	High
Ticarcillin R	93.1 (3.4)	96.2 (3.9)	86.9 (2.5)	100.7 (3.5)	99.3 (2.5)	97.7 (3.5)
Ticarcillin S	94.1 (4.7)	94.9 (2.9)	88.7 (2.8)	101.0 (3.2)	98.9 (2.5)	99.5 (2.9)
Clavulanate	96.4 (3.7)	92.3 (3.1)	93.7 (4.0)	99.1 (3.8)	98. 4(2.9)	97.8 (3.7)

Note: Percentage relative standard deviations are presented in parentheses.

Pharmacokinetics. The log concentration–time curves of ticarcillin and clavulanate are presented in Figure 6. Pharmacokinetic parameters such as C_{max}, t_{max}, $t_{1/2}$, and $AUC_{0\text{-}\infty}$ are listed in Table 6. The results revealed that the pharmacokinetic parameters including the peak values appeared rapidly *in vivo* after intravenous administration in rats. However, the drug–time curves of the two components were different in rats. The plasma concentration of ticarcillin S was 8760 ± 395 ng/mL, compared with 8,740,000 ± 875,386 ng/mL for ticarcillin R. The plasma concentration of

Table 4. Short- and long-term stability of ticarcillin and clavulanate in rat plasma.

		Short-term stability		Long-term stability	
Compounds	Concentration (ng/mL)	Concentration (ng/mL)	Accuracy (%)	Concentration (ng/mL)	Accuracy (%)
Ticarcillin R	30	30.4 ± 0.6	1.3	28.7 ± 0.7	−4.3
	2400	2349 ± 15	−2.1	2388 ± 17	−0.5
	8000	7918 ± 107	−1.0	8061 ± 113	0.8
Ticarcillin S	30	28.8 ± 0.9	−4.0	29.3 ± 1.1	−2.3
	2400	2457 ± 19	2.4	2431 ± 14	1.3
	8000	7931 ± 113	−0.9	7956 ± 121	−0.6
Clavulanate	80	81.4 ± 0.6	1.8	82.6 ± 1.2	3.2
	300	305 ± 11	1.7	289 ± 17	−3.7
	2400	2389 ± 118	−0.5	2373 ± 109	−1.1

Table 5. Freeze–thaw and extracted sample stabilities of ticarcillin and clavulanate in rat plasma.

		Freeze-thaw stability		Extracted sample stability	
Compounds	Concentration (ng/mL)	Concentration (ng/mL)	Accuracy (%)	Concentration (ng/mL)	Accuracy (%)
Ticarcillin R	30	29.4 ± 1.4	−2.0	28.6 ± 0.8	−4.7
	2400	2439 ± 10	1.6	2427 ± 16	1.1
	8000	8078 ± 97	1.0	8291 ± 91	3.6
Ticarcillin S	30	31.5 ± 1.7	5.0	30.8 ± 1.3	2.7
	2400	2344 ± 16	−2.3	2,465 ± 12	2.7
	8000	7961 ± 95	−0.5	7985 ± 93	−0.2
Clavulanate	80	83.1 ± 0.8	3.9	77. 8 ± 1.0	−2.8
	300	291 ± 15	−3.0	295 ± 17	−1.7
	2400	2412 ± 92	0.5	2344 ± 98	−2.3

clavulanate was 1656 ± 667 ng/mL at 48 hours after administration. The area under the curve of the plasma concentration of ticarcillin S was 3270 ng·hours/mL, compared with 3,654,298 ng·hours/mL for ticarcillin R. The area under the curve of the plasma concentration of clavulanate was 541 ng·hours/mL. Furthermore, $t_{1/2}$ of ticarcillin S was 0.21 hour, and the clearance and apparent volume of distribution were 49.07 L/hour/kg and 12.2 L/kg, respectively. Conversely, $t_{1/2}$, clearance, and the apparent volume of distribution for ticarcillin R were 1.04 hours, 0.044 L/hour/kg, and 0.02 L/kg, respectively. $t_{1/2}$ of clavulanate was 0.17 hour, and the clearance and apparent volume of distribution were 347 L/hour/kg and 66.7 L/kg, respectively. Based on these results, we speculate that clavulanate has the shortest $t_{1/2}$ and most rapid elimination. These findings indirectly demonstrated that the pharmacokinetic characteristics of the two isomers of ticarcillin were different *in vivo*.

Discussion

Ticarcillin sodium–clavulanate is a compound preparation consisting of β-lactam antibiotics and β-lactamase inhibitors.

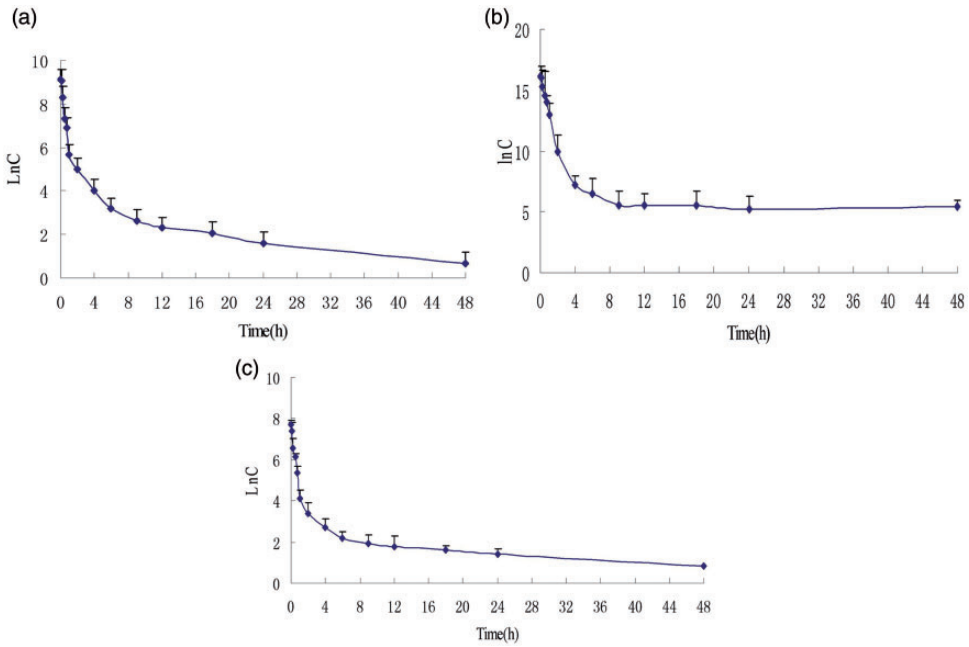

Figure 6. Log concentration–time curves of ticarcillin and clavulanate in rats after intravenous administration of the compound preparation. (a) Ticarcillin S. (b) Ticarcillin R. (c) Clavulanate.

Ticarcillin is a novel thienyl carboxyl penicillin and semi-synthetic anti-*Pseudomonas* compound. Potassium clavulanate is a strong inhibitor of various β-lactamases. Therefore, the compound preparation is a distinct antibiotic with strong antibacterial activity and a broad antibacterial spectrum. This drug has exhibited good efficacy in elderly patients with CAP. In addition, low rates of resistance have been identified for NFGNB. To understand the properties of this drug, we examined the pharmacokinetic characteristics of ticarcillin and clavulanate in rats. The pharmacokinetic parameters obtained in this study should have great significance for clinical practice.

In this study, biological samples were pre-treated to remove interfering substances, thereby guaranteeing good recovery of the test object. Proper plasma sample treatment resulted in high extraction recovery and low matrix effects. Meanwhile, we compared the two isolation methods (direct precipitation of organic solvents and liquid–liquid extraction). Methanol, acetonitrile, and acetone solvents were studied to identify the best approach that could effectively precipitate proteins and concentrate sample. The results confirmed the advantages of direct protein precipitation using acetonitrile, including easier operation, the absence of matrix effect, low background noise, high extraction efficiency, easy sample preparation, and suitability for biological analysis. Therefore, this method was selected for further analysis. The optimum extraction conditions were determined by adjusting the vortex mixing time (3, 5, and 10 minutes), centrifugation speed (3000, 4000, and 5000 rpm), and centrifugation temperature (5°C and room temperature). The results suggested the vortex mixing for 5 minutes and centrifugation at 4000

Table 6. Pharmacokinetic parameters of ticarcillin and clavulanate in rat plasma after intravenous administration.

Analytes	C_{max}[a] (ng/mL)	t_{max} (h)	$t_{1/2}$[a] (h)	AUC0-t[a] (ng·h/mL)	AUC0-∞[a] (ng·h/mL)	CL(/F)[a] (L/h/kg)	Vss obs[a] (L/kg)
Ticarcillin R	8,740,000 ± 875,386	0.083	1.04 ± 0.09	3,654,298 ± 430,890	3,654,643 ± 431,153	0.044 ± 0.01	0.02 ± 0.00
Ticarcillin S	8760 ± 395	0.083	0.21 ± 0.01	3270 ± 262	3274 ± 264	49.67 ± 3.79	12.2 ± 0.68
Clavulanate	1656 ± 667	0.083	0.17 ± 0.04	541 ± 254	559 ± 251	347 ± 204	66.7 ± 27.1

[a]Mean ± standard deviation.
AUC, area under the curve; h, hour; CL(/F), clearance; Vss obs, observed volume of distribution at steady state.

rpm and 5°C for 10 minutes were suitable for biological sample analysis.

We have compared methanol–water and acetonitrile–water mobile phase systems. The results indicated that the acetonitrile–water system is superior to the methanol–water system. In addition, three additives, namely formic acid (0.05%, 0.1%, and 0.5%), acetic acid (0.05%, 0.1%, and 0.5%), and ammonium acetate (0.05%, 0.1%, and 0.5%), were selected to identify the best mobile phase. The results indicated that 0.1% formic acid had several advantages including ionization promotion, resolution improvement, higher detection sensitivity, and less endogenous substance interference. Therefore, 0.1% formic acid was added to the aqueous and organic layers of the mobile phase in this study. In addition, we employed a Waters UPLC system together with a SCIEX API4000 QTrap triple quadrupole mass spectrometer. A Waters ACQUITY BEH C18 column (50 mm × 2.1 mm, 1.7 μm) was used to separate both components and the internal standard in a short analysis time. There was no need for a nitrogen stream during sample pre-treatment. Hence, the entire operation was simpler. With the simple protocol, we demonstrated that the analytical result is accurate and reliable and reflects the actual situation of the sample.

In *in vivo* assays, suitable internal standards are critical for assay analysis. Tolbutamide has a relatively large response value in the positive ion mode. Its peak time is similar to the analytes of interest. Based on these considerations, tolbutamide was selected as the internal standard. Meanwhile, two isomers of ticarcillin and clavulanate could be ionized under both the positive and negative ion modes. However, the response value of ticarcillin under the positive ion mode was higher than that under the negative ion mode. The response value of the internal standard was similar under the positive and negative

ion modes. Therefore, we obtained the best ionization efficiency and higher sensitivity under the positive ion mode.

Conclusions

We have constructed a highly sensitive and selective UPLC-ESI-MS/MS analysis method, which was successfully applied to quantify the levels of ticarcillin and clavulanate in rat plasma. The method has several strengths, including a simple preparation process, short analysis time, and accurate and reliable results. This method could both be applied to ticarcillin and clavulanate measurement in rat plasma but used to examine traces of ticarcillin and clavulanate in other biological samples. The method provides a new experimental method for *in vivo* analysis of ticarcillin and clavulanate.

Author contributions

MLW: Guarantor of the integrity of the entire study, responsible for study concepts & design, experimental studies, data & statistical analysis, manuscript preparation & editing. LTZ: Study concepts & design, manuscript review. JSC: Definition of intellectual content. WZP: Literature review. XLL: Clinical research. QW: Experimental studies. JZ: Data acquisition & analysis. All authors approved the final version of this manuscript.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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ORCID iD

Lantong Zhang https://orcid.org/0000-0003-1765-2948

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