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LC-MS/MS quantification of omadacycline in human plasma for therapeutic drug monitoring: method development and clinical application

Yueyuan Wang, Zhipeng Diao, Yuchen Qu, Kai Fan, Chen Feng, Bo Lv, Yunli Yu ✉ & Zhou Geng ✉

Omadacycline, a third-generation tetracycline antibiotic, exhibits time-dependent pharmacokinetics. The ratio of the 24-hour area under the concentration-time curve to minimum inhibitory concentration (AUC_{0-24}/MIC) serves as the primary pharmacokinetic/pharmacodynamic (PK/PD) index for omadacycline, demonstrating strong correlation with therapeutic efficacy, particularly in critically ill patient populations. To facilitate routine therapeutic drug monitoring (TDM) in clinical practice, a high-performance liquid chromatography - tandem mass spectrometry (HPLC-MS/MS) method was developed for quantification of omadacycline in human plasma. An Agilent 1260 series liquid chromatograph and an API 4000 triple tandem quadrupole mass spectrometer were used for the determination of omadacycline and endocannabinoids. The separation was performed on a Phenomenex KINETEX XB-C18 column (2.6 µm, 3 × 50 mm) with 0.1% formic acid-water and pure acetonitrile as the mobile phases. The LC-MS/MS separation was carried out using a gradient elution procedure at a flow rate of 0.4 mL/ min, and the total run time was 5 min. An electrospray ionization (ESI) was selected, and the detection ions of omadacycline and the internal standard (fexofenadine-d6) were determined by mass spectrometry scanning with multiple reaction monitoring (MRM) in positive ion mode as follows: m/z 557.4→453.4 and m/z 508.4→472.8, respectively. The established linear range (20-2000 ng/mL) effectively covers the plasma concentration range encountered in >98% of clinical samples, making it suitable for routine therapeutic drug monitoring. The method demonstrated acceptable selectivity, recovery, and matrix effect. The results of intra-day precision and inter-day precision show that the relative standard deviation (RSD) is less than 10%, while the relative error (RE) is within ±10.00%. In this study, we developed and validated a simple, sensitive and accurate method to quantify the concentration of omadacycline in human plasma using LC-MS/MS. This offers essential technical support for the rational clinical application of omadacycline.

Keywords Omadacycline, Plasma, Therapeutic drug monitoring, LC-MS

Omadacycline is a novel tetracycline antibacterial agent approved by the United States Food and Drug Administration (FDA) for the treatment of community-acquired pneumonia and acute bacterial skin and skin structure infections[1]. As a semi-synthetic compound related to minocycline, omadacycline exhibits the unique ability to evade widespread efflux and target-protective antimicrobial resistance mechanisms, and it demonstrates broad-spectrum antibacterial activity against a wide range of bacterial pathogens[2]. Tetracyclines are time-dependent antibiotics, characterized by a prolonged post-antibiotic effect. The ratio of the area under the 24-hour plasma concentration-time curve to the minimum inhibitory concentration ($AUC_{0\sim24h}$/MIC) is a key pharmacokinetic/pharmacodynamic (PK/PD) parameter that correlates strongly with therapeutic efficacy[3].

Current pharmacokinetic studies of omadacycline have primarily been conducted in healthy subjects[4] and in several specific populations, including those with renal impairment[5]hepatic impairment[6,7] and co-morbidities[8]. The pharmacokinetic profile of omadacycline remains relatively consistent across patients with different

Department of Pharmacy, The Second Affiliated Hospital of Soochow University, Suzhou 215004, Jiangsu, China. ✉email: haoyyl0902@163.com; gengzhouaa@163.com

ages, genders, races, body weights, renal function, or hepatic function[9]. However, in critically ill patients, the pharmacokinetic parameters of drugs are often altered due to the complexity of their clinical conditions, the variability in their baseline states, the extent of organ dysfunction, and the presence of life-supporting therapies[10–15]. Given these complexities, therapeutic drug monitoring (TDM) of omadacycline in critically ill patients is essential.

Liquid chromatography-mass spectrometry (LC-MS) methods are currently the gold standard for quantitative detection and are increasingly being utilized in precision medicine[16]. However, there is a scarcity of quantitative methods available for omadacycline. To date, only a few studies have established quantitative detection of omadacycline in plasma[17,18] and feces[19]. In this study, we aim to develop a rapid LC-MS method for the quantification of omadacycline, characterized by high sensitivity, high selectivity, and simple sample pre-treatment. Building on the existing methods, this new method is expected to be applicable to clinical detection, thereby providing technical support for clinical TDM. Additionally, it will lay the foundation for future PK/PD-related real-world studies.

Materials and experimental section

Instruments and equipment

High performance liquid chromatography (HPLC) was performed on an Agilent 1260 HPLC system (Agilent, USA) in tandem with an API-4000 Triple Quadrupole Tandem Mass Spectrometer (SCIEX, USA). The data processing system used was Analyst 1.4.2 (SCIEX, USA).

Reagents

Omadacycline benzenesulfonate (purity ≥ 98%) and fexofenadine-d6 (internal standard, purity ≥ 98%) were purchased from Aladdin(China). Methanol, acetonitrile, and formic acid (all HPLC grade) were purchased from Merck KGaA (Germany).

Standard working solutions and quality control samples

Prepare a stock solution of omadacycline at a concentration of 370 μg/mL using dimethyl sulfoxide (DMSO) as the solvent. Subsequently, dilute this stock solution with acetonitrile to obtain standard curve working solutions with concentrations of 200, 500, 1000, 2000, 5000, 10,000, and 20,000 ng/mL. Additionally, prepare quality control working solutions with concentrations of 400, 4000, and 15,000 ng/mL using the same dilution method. In parallel, prepare a stock solution of fexofenadine-d6 at a concentration of 1.0 mg/mL using pure acetonitrile as the solvent for the internal standard. All prepared stock solutions should be stored at −80 °C.

Sample Preparation

The internal standard stock solution (fexofenadine-d6, 1.0 mg/mL) was diluted with pure acetonitrile to a concentration of 50 ng/mL. This solution was used as a protein precipitant for biological sample processing and was freshly prepared at the beginning of each experiment.

Transfer 5 μL of each omadacycline standard curve working solution and quality control (QC) working solution into separate 1.5 mL centrifuge tubes, then add 45 μL of blank plasma to each tube. Vortex the mixtures for 15 s to ensure homogeneity, yielding standard curve samples (S1–S7) at concentrations of 20, 50, 100, 200, 500, 1000, and 2000 ng/mL, along with low (40 ng/mL), medium (400 ng/mL), and high (1500 ng/mL) QC samples.

Next, add 200 μL of the protein precipitant to 50 μL of clinical samples, standard curve samples, and quality control samples. Precipitate the proteins by shaking for 5 min, followed by centrifugation at 13,000 rpm for 5 min at 4 °C. Transfer 50 μL of the supernatant to 200 μL of 0.1% formic acid (FA) in water, vortex and mix well. Finally, transfer 100 μL of the supernatant to an injection vial and inject 5 μL of the supernatant for analysis.

Chromatographic and mass spectrometric conditions

The chromatographic separation was performed on a Phenomenex KINETEX XB-C18 column (3.0 × 50 mm, 2.6 μm) with a Phenomenex XB-C18 guard column (3.0 mm). The mobile phase consisted of an organic phase (B: acetonitrile) and an aqueous phase (A: water containing 0.1% FA). The column temperature was maintained at 40 °C, and the flow rate was set at 0.4 mL/min. The injection volume was 5 μL, and the total analysis time for each sample was 5 min. The gradient elution procedure is detailed in Table 1.

For mass spectrometry, an electrospray ionization (ESI) source was employed in positive ion mode with multiple reaction monitoring (MRM). The detected ions for omadacycline and the internal standard were m/z 557.4 → 453.4 and m/z 508.4 → 472.8, respectively.

Time	A: 0.1% FA-Water	B: ACN
0	90	10
0.5	90	10
1.5	3	97
3.0	3	97
3.5	90	10
5.0	90	10

Table 1. Gradient elution program.

Method validation

Selectivity and specificity

In this study, we conducted a selectivity assessment using blank plasma matrices obtained from six drug-free volunteers. The primary objective was to confirm the absence of interfering peaks within the retention region of the analyte and in the endogenous matrix. For each batch of matrix, one blank sample and one lower limit of quantification (LLOQ) sample were prepared, resulting in a total of twelve samples. The criteria for matrix interference and inter-analyte interference were set as follows: the interference at the analyte retention times should not exceed 20% of the LLOQ response or 5% of the mean response of the LLOQ samples and QC samples.

Linearity and lower limit of quantification

A calibration curve was constructed by performing linear regression analysis on the ratio of the analyte omadacycline peak area to the internal standard peak area versus the known concentration of the analyte, using seven calibration standards. The accuracy of the mean of LLOQ samples in each analysis batch should be within ± 20%, and the signal-to-noise ratio (SNR) of LLOQ should be at least greater than 10.

Accuracy and precision

Precision was assessed using four concentration levels (LLOQ, LQC, MQC, and HQC), with six replicates per concentration level, across at least three batches within one week to evaluate inter-day accuracy and precision. Precision is a measure of the closeness between repeated measurements, expressed as relative standard deviation (RSD). Accuracy is the percentage difference between a measured value and a nominal value, expressed as relative error (RE). The acceptance criteria for RE are ± 20% for LLOQ samples and ± 15% for other QC samples. The acceptance criteria for RSD are ≤ 20% for LLOQ samples and ≤ 15% for other QC samples.

Carryover, recovery and matrix effects

Carryover refers to the residual material remaining in the system after the injection of the highest concentration of the standard curve. The acceptable standard for residue is that the peak area at the analyte position should be less than 20% of the peak area of the analyte in the LLOQ sample, and the peak area at the internal standard position should be less than 5% of the peak area of the internal standard.

The extraction recovery of the analyte was calculated by dividing the peak area of the analyte recovered from the biological matrix by the peak area of the analyte without extraction. The recovery was assessed using QC samples at three concentration levels, with six replicates for each concentration. The acceptance criterion was that the extraction recovery of the analyte should be similar across different QC concentrations.

Matrix effects were evaluated using three QC concentration levels, with six replicates per concentration level. Six batches of blank plasma samples from healthy individuals obtained from different sources were processed. For each batch, the matrix effect factors for the analyte and the internal standard were calculated by determining the ratio of the peak area in plasma to the peak area in pure acetonitrile. These factors were then normalized using the internal standard. The acceptance criteria for matrix effects stipulate that the coefficient of variation should not exceed 15% at three QC concentration levels.

Stability

The stability of omadacycline was evaluated by analyzing QC samples at three QC concentration levels under various storage and processing conditions. For each concentration level and each condition, six samples were prepared. The short-term stability assessment included the following conditions: 4 h at room temperature, 24 h in the autosampler, 48 h in the autosampler, 24 h of storage at 4 °C, and three freeze-thaw cycles (from − 80 °C to room temperature). Long-term stability was evaluated by storing the samples at −80 °C for 217 days. The measured concentrations at each level were required to be within 85–115% of the nominal concentration, and the RE was not permitted to exceed 15.0%.

Clinical application

A validated method was employed to monitor the levels of omadacycline in the plasma of patients hospitalized for pulmonary infections. The dosing regimen for these patients involved an initial intravenous administration of 0.2 g of omadacycline, followed by a maintenance dose of 0.1 g once daily. This study collected samples from hospitalized patients in the respiratory department of our hospital after they had received intravenous infusion of omadacycline, using EDTA blood collection tubes. These samples were then centrifuged at 2000 g for 10 min, and the plasma supernatant was carefully collected and stored at −80 °C for subsequent mass spectrometry analysis. The study was approved by the Institutional Ethics Committee of the Second Affiliated Hospital of Soochow University.

Results

Development of methods

For the determination, we employed an electrospray ionization source in conjunction with multi-reactive ion monitoring and positive ion mode as the mass spectrometry conditions. Fexofenadine-d6 was selected as the internal standard due to its similar molecular weight, chemical properties, retention behavior, ionization, and extraction efficiency compared to omadacycline. The quantitative ion pairs were selected as m/z 557.4→453.4 for omadacycline and m/z 508.4→472.8 for the Fexofenadine-d6. The mass spectrometry parameters were meticulously optimized to achieve the highest sensitivity and reproducible fragmentation patterns. Omadacycline is a weakly polar compound, therefore, this study employed a reversed-phase column for separation. During method development, various mobile phase compositions and ratios were tested to identify the optimal conditions. In the end, omadacycline and internal standard exhibited ideal retention time and good

peak shape when the aqueous phase was 0.1% FA-water and the organic phase was pure acetonitrile, ensuring reliable quantification. The sensitivity of omadacycline was enhanced by adding formic acid to the mobile phase. HPLC separation was performed using a gradient elution program at a flow rate of 0.4 mL/min.

During the sample pretreatment method development, we initially employed protein precipitation (plasma: precipitant = 1:3), but encountered significant matrix effects. To reduce matrix effects, we enhance the proportion of the aqueous phase in the supernatant after protein precipitation and to closely match it to the mobile phase, the supernatant was diluted by adding formic acid (0.1% v/v in water) after protein precipitation (plasma: precipitant = 1:4)[18]. This approach effectively improved peak shapes and minimized matrix interferences. Following optimization, all analytes eluted at shorter retention times, with a total run time of 5.0 min.

Method validation

Selectivity and specificity

The results showed that the retention times of omadacycline and fexofenadine-d6 were approximately 2.78 min and 3.19 min, respectively. For omadacycline, the ratio of the response value of any interfering peak to the response value of the lower limit of quantification (LLOQ) was within the range of 2.7–5.1%. Meanwhile, the ratio of the response value of any interfering peak at the retention time of the internal standard to the response value of the fexofenadine-d6 itself was within the range of 0.1–0.3%. As shown in Fig. 1, no significant interfering peaks were detected at the retention times for omadacycline and fexofenadine-d6. These findings demonstrate that the method exhibits high selectivity and specificity, enabling accurate detection and identification of omadacycline in complex plasma samples without significant interference from other components.

Linearity and lower limit of quantification

A linear least squares regression analysis was performed for omadacycline and fexofenadine-d6 by plotting the ratio of peak area to concentration, with a weighting factor of $1/X^2$. omadacycline was found to be linear over the concentration range of 20 to 2000 ng/mL, with correlation coefficients exceeding 0.99 ($r^2 = 0.9988$). As shown in Fig. 2, the classic linear regression equation is $y = 0.00617x - 0.0134$ ($r = 0.9988$). The LLOQ sample used in this study had a signal-to-noise ratio of 90 under this method, indicating adequate sensitivity for the detection of omadacycline in the specified concentration range.

Accuracy and precision

The intra- and inter-day precision of omadacycline was evaluated by analyzing six replicate spiked samples at four concentration levels. As shown in Table 2, the intra-day precision of omadacycline ranged from 1.99 to 5.24%, with an accuracy of −1 to −5%. Meanwhile, the inter-day precision ranged from 2.81 to 4.1%, with an accuracy of −6.58 to −2.10%. These results These results met FDA bioanalytical method validation guidelines, indicating that the method is robust and reliable for the analysis of omadacycline.

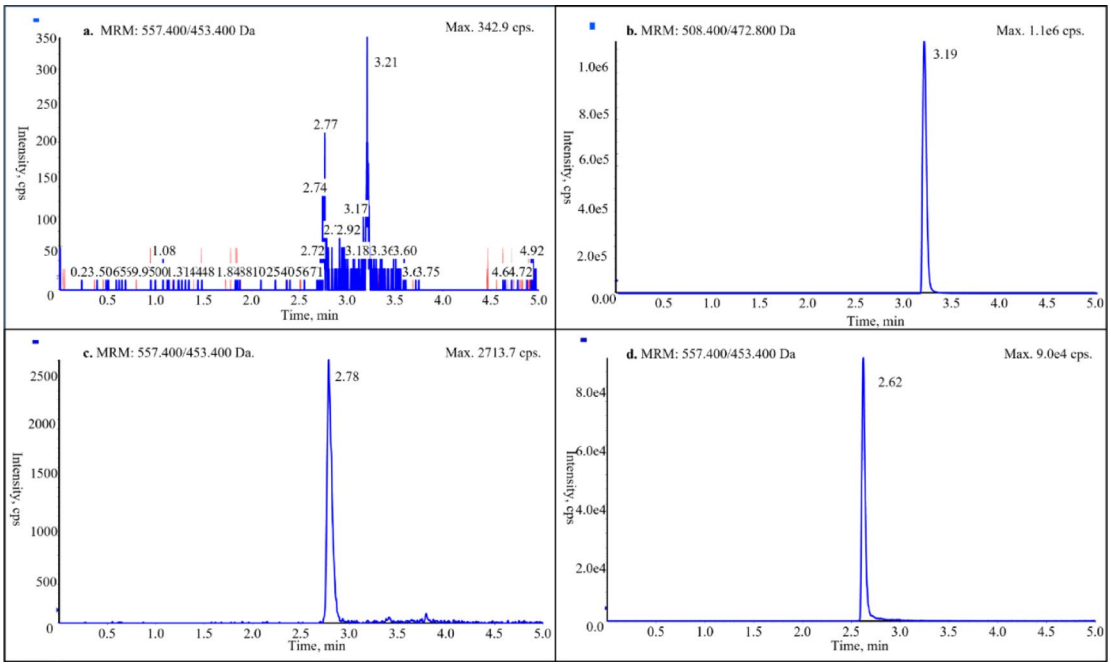

Fig. 1. Chromatograms of blank human plasma (**a**), internal standard (**b**), LLOQ (**c**), and clinical plasma sample (**d**).

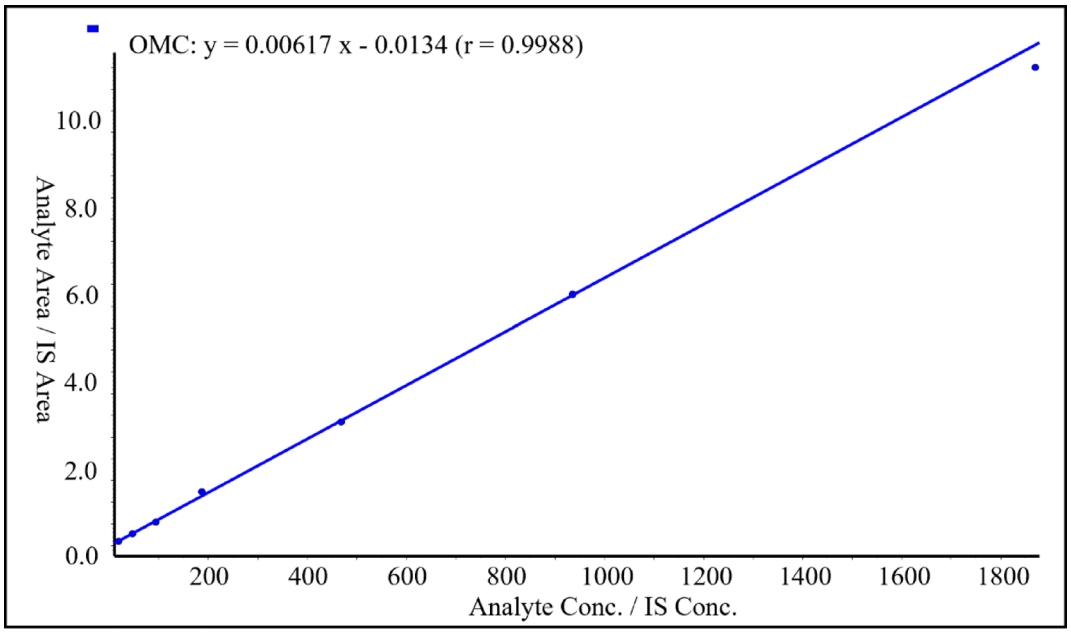

Fig. 2. The linear regression equations of omadacycline.

QC Level	Nominal Concentration (ng/mL)	Intra-day ($n=6$)		Inter-day ($n=18$)	
		Precision (RSD, %)	Accuracy (RE, %)	Precision (RSD, %)	Accuracy (RE, %)
LLOQ	20	5.24	−3.08	4.1	−6.58
LQC	40	3.63	3.08	2.81	0.67
MQC	400	1.99	5	3.63	2.10
HQC	1500	2.82	−1	3.02	−3.59

Table 2. The intra- and inter-day accuracy and precision of omadacycline.

QC Level	Carryover ($n=6$)		Matrix effects ($n=6$)	
	Mean(%)	RSD(%)	Mean(%)	RSD(%)
LQC	86.70	4.37	100.10	4.35
MQC	96.26	2.44	96.32	1.74
HQC	97.08	2.83	105.56	7.45

Table 3. Recovery and matrix effects of omadacycline.

Carryover, recovery and matrix effects

The interference peak area at the analyte position in the blank plasma sample was 6.39% of the LLOQ peak area, while the interference peak area at the internal standard position was 1.56% of the internal standard peak area. These values satisfy the acceptance criteria for carryover experiments, demonstrating that the analytical method effectively minimizes carryover.

The recoveries and matrix effects of omadacycline were determined and calculated using 3 concentrations of QC samples. The results showed that the recoveries of the three concentrations of omadacycline ranged from 86.7 to 97.08%, with the average recovery of 93.35%, and the average recovery of the internal standard was 98.47%. The matrix effects were all within ± 10%, and was no significant difference in the matrix effects between the three concentrations of QC samples, which did not affect the assay, as shown in Table 3.

Stability

The RSD and RE of omadacycline in the three concentrations of QC samples under different storage conditions were within ± 15%. These results indicate that the samples maintained good short-term and long-term stability under the examined conditions, thereby supporting the accuracy of the test results and meeting the requirements for clinical testing. The detailed results are presented in Table 4.

Stability Conditions	LQC ($n=6$)		MQC ($n=6$)		HQC ($n=6$)	
	RSD(%)	RE(%)	RSD(%)	RE(%)	RSD(%)	RE(%)
Room temperature, 4 h	3.31	3.31	1.71	8.82	7.83	−8.00
4℃ Refrigerator, 24 h	5.92	5.92	2.77	−4.46	1.90	−6.47
autosampler 24 h	5.64	5.64	2.77	2.18	3.63	−10
autosampler 48 h	3.08	3.08	4.50	−0.11	3.59	−4.13
freeze-and-thaw, −80℃, 3 cycles	5.51	5.51	2.43	1.54	2.45	−7.50
Freezer (−80 oC), 217 day	5.51	5.51	2.43	−5.06	1.74	−5.80

Table 4. Stability data of Omadacycline under different storage conditions.

	All ($n=54$)	Man ($n=37$)	Woman ($n=17$)
Age (years)	69.37 ± 13.88	70.76 ± 10.56	66.35 ± 18.85
BMI (kg)	21.70 ± 4.15	21.82 ± 3.77	21.43 ± 4.87
Hypertension	36	27	9
Diabetes	12	9	3
Smoking	14	13	1
Drinking	9	9	0
Severe pneumonia	14	14	0

Table 5. Basic clinical information of the patient.

Clinical application

After undergoing rigorous validation, the LC-MS method for detecting omadacycline has been successfully applied to the testing of clinical samples. A total of 127 samples were collected from 54 patients, and omadacycline concentration tests were conducted on these samples according to standard sample processing procedures. Among the 54 patients with severe pneumonia, the majority were elderly males, reflecting the higher incidence rate in men compared to women. Detailed clinical characteristics are presented in Table 5. The plasma concentration results of omadacycline were categorized into trough concentration, peak concentration, and other random concentration. The trough concentration distribution of omeprazole in patients is 243.15 ± 124.74 ng/mL, the peak concentration distribution is 1,181.17 ± 726.57 ng/mL, and the concentration distribution of other random concentration is 600.27 ± 303.73 ng/mL. The visualization of concentration results is shown in Fig. 3. The detailed information and corresponding plasma drug concentration data for the clinical patients are provided in supplementary files.

Discussion

Determination of blood drug concentration in vivo by LC-MS/MS can obtain a single peak of the drug in the complex components of blood samples, which has the advantages of high sensitivity and specificity, and therefore is widely used in the field of drug analysis. To explore the drug concentration level of omadacycline in the plasma of clinical patients, the LC-MS/MS method established in this study was used with acetonitrile as the protein precipitant. The chromatographic conditions were optimized using 0.1% formic acid in water to improve peak shape. Additionally, this study employed a 50 mm C18 reverse-phase chromatography column to achieve a faster analysis time for omadacycline while obtaining a more ideal retention time. Finally, the method met the requirements for the LLOQ of detection without interference from impurities, and demonstrated high accuracy, precision, minimal matrix effects, and high extraction recoveries, thus fulfilling the requirements for clinical samples. We are developing comprehensive standard operating procedures (SOPs). After a period of clinical sample verification, this method can be applied to TDM for omadacycline.

The results of plasma omadacycline concentration in 127 clinical patients showed that the linear range could cover the plasma concentration of patients after intravenous omadacycline infusion. Under the same dosing regimen, the mean peak concentration in healthy Chinese adults has been reported to be around 1,990 ng/mL[20]. In the present study, the mean plasma omadacycline concentration in patients was slightly lower than the reported value, and there was a large variation in plasma concentrations among patients. This may be attributed to the fact that the study population consisted of elderly patients with severe pneumonia, most of whom had multiple underlying diseases and complex conditions. In addition, studies have found that the plasma concentration of omadacycline in women is higher than that in men, while the majority of this study was conducted on elderly men[9]. Additionally, many uncontrollable factors in real-world research, such as diet[21].

The limitations of this study stem from the difficulty in obtaining standard samples and the absence of an isotopic internal standard for omadacycline. Additionally, there was no experimental design or intervention in the clinical medication regimen. Although the medication regimen for clinical patients was essentially the same, differences in the timing of drug administration among patients may have led to variations in plasma drug concentrations. To date, there have been few studies on the plasma concentration of omadacycline, and a clear reference range for plasma omadacycline concentration has not been established. Currently, only

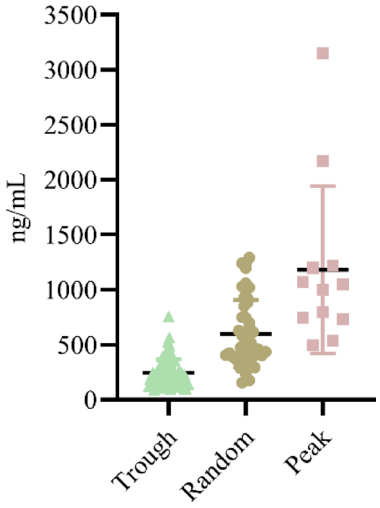

Fig. 3. The plasma concentration distribution of omadacycline.

a few animal experiments have explored the AUC/MIC target values of omadacycline for several strains of community-acquired pneumonia[22–24]. Exploring the effective target value reference range of omadacycline will likely be an important research direction in the future. We will continue to focus on the clinical application of omadacycline, conduct real-world research on its use, and explore pharmacodynamics indexes in patients with severe pneumonia.

Conclusion

The LC-MS method established in this study can rapidly detect the concentration of omadacycline in patients' plasma within five minutes of spectrometry analysis, featuring a simple and easy-to-perform sample pre-treatment procedure. This capability provides robust technical support for TDM and the rational clinical use of omadacycline, thereby laying the foundation for future real-world research on omadacycline.

Data availability

All data generated in this study are included in this manuscript and its supplementary files.

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References

1. Markham, A. & Keam, S. J. Omadacycline: first global approval. *Drugs* **78** (18), 1931–1937 (2018).
2. Durães, F. & Sousa, E. Omadacycline: a newly approved antibacterial from the class of tetracyclines. *Pharmaceuticals* **12** (2), 63 (2019).
3. Yaghoubi, S. et al. Tigecycline antibacterial activity, clinical effectiveness, and mechanisms and epidemiology of resistance: narrative review. *Eur J Clin Microbiol Infect Dis.* **41** (7), 1003–1022 (2022).
4. Gotfried, M. H. et al. Comparison of Omadacycline and Tigecycline pharmacokinetics in the plasma, epithelial lining fluid, and alveolar cells of healthy adult subjects. *Antimicrob. Agents Chemother.* **61** (9), 1135–1117. https://doi.org/10.1128/AAC.01135-17 (2017).
5. Berg, J. K. et al. Pharmacokinetics and safety of Omadacycline in subjects with impaired renal function. *Antimicrob Agents Hemother*. **62** (2), 2057–2017. https://doi.org/10.1128/AAC.02057-17 (2018).
6. Kovacs, S. J. et al. An open-label study of the impact of hepatic impairment on the pharmacokinetics and safety of single oral and intravenous doses of Omadacycline. *Antimicrob Agents Chemother*. **64** (11), 1650–1620. https://doi.org/10.1128/AAC.01650-20 (2020).
7. Zhang, A. et al. Physiologically based Pharmacokinetic model for predicting Omadacycline Pharmacokinetics and pharmacodynamics in healthy and hepatic impairment populations. *Clin Ther*. **46** (8), 629–635 (2024).
8. Trang, M. et al. Evaluation of the impact of comorbidities on Omadacycline pharmacokinetics. *Antimicrob Agents Chemother*. **67** (4), 239721. https://doi.org/10.1128/aac.02397-21 (2023).
9. Wang, K. et al. Evaluation of Omadacycline dosing regimens in Chinese using population pharmacokinetic-pharmacodynamic analysis. *Eur J Pharm Sci*. **195**, 106713 (2024).
10. Annoni, F., Grimaldi, D. & Taccone, F. S. Individualized antibiotic therapy in the treatment of severe infections. *Expert Rev Anti Infect Ther*. **18** (1), 27–35 (2020).
11. Taccone, F. S. et al. Insufficient β-lactam concentrations in the early phase of severe sepsis and septic shock. *Critical Care*. **14** (4), R126 (2010).
12. Jacobs, A. et al. β-Lactam dosage regimens in septic patients with augmented renal clearance. *Antimicrob Agents Chemother*. **62** (9), 2534–2517. https://doi.org/10.1128/AAC.02534-17 (2018).
13. Beumier, M. et al. β-Lactam antibiotic concentrations during continuous renal replacement therapy. *Critical Care*. **18** (3), R105 (2014).
14. Joukhadar, C. et al. Impaired target site penetration of beta-lactams May account for therapeutic failure in patients with septic shock. *Critical Care Medicine*. **29** (2), 385–391 (2001).

15. Donadello, K. et al. β-Lactam pharmacokinetics during extracorporeal membrane oxygenation therapy: A case-control study. *Int J Antimicrob Agents*. **45** (3), 278–282 (2015).
16. Gaspar, V. P., Ibrahim, S., Zahedi, R. P. & Borchers, C. H. Utility, promise, and limitations of liquid chromatography-mass spectrometry-based therapeutic drug monitoring in precision medicine. *J Mass Spectrom*. **56** (11), 4788. https://doi.org/10.1002/jms.4788 (2021).
17. Flarakos, J. et al. Clinical disposition, metabolism and in vitro drug-drug interaction properties of Omadacycline. *Xenobiotica* **47** (8), 682–696 (2017).
18. Suhang, G. et al. Development, validation, and clinical application of a UPLC-MS/MS method for Omadacycline determination in human serum. *J Pharmacol Toxicol Methods*. **127**, 107503 (2024).
19. Hu, C., Wang, W., Jo, J. & Garey, K. W. Development and validation of LC-MS/MS for quantifying Omadacycline from stool for gut Microbiome studies. *J Chromatogr B Analyt Technol Biomed Life Sci*. **1236**, 124057 (2024).
20. Yang, H. et al. Pharmacokinetics, safety and pharmacokinetics/pharmacodynamics analysis of Omadacycline in Chinese healthy subjects. *Front Pharmacol*. **13**, 869237 (2022).
21. Tzanis, E. et al. Effect of food on the bioavailability of Omadacycline in healthy participants. *J Clin Pharmacol*. **57** (3), 321–327 (2017).
22. Lepak, A. J., Zhao, M., Marchillo, K., VanHecker, J. & Andes, D. R. In vivo pharmacodynamic evaluation of Omadacycline (PTK 0796) against Streptococcus pneumoniae in the murine pneumonia model. *Antimicrob Agents Chemother*. **61** (5), 2368–2316. https://doi.org/10.1128/AAC.02368-16 (2017).
23. Rodvold, K. A. & Pai, M. P. Pharmacokinetics and pharmacodynamics of oral and intravenous Omadacycline. *Clin Infect Dis*. **69** (Suppl 1), S16–S22 (2019).
24. Lepak, A. J., Zhao, M., Marchillo, K., VanHecker, J. & Andes, D. R. In vivo pharmacodynamics of Omadacycline against Staphylococcus aureus in the neutropenic murine thigh infection model. *Antimicrob. Agents Chemother.* **63(7)**, 624–619. https://doi.org/10.1128/AAC.00624-19 (2019).

Author contributions
Zhou Geng and Yunli Yu designed the study and conducted experimental operations, Yueyuan Wang performed the statistical analysis and wrote the manuscript. Zhipeng Diao carried out the experiments. Bo Lv and Chen Feng collected clinical samples, Yuchen Qu and Kai Fan conducted clinical sample processing, and Yueyuan Wang conducted clinical sample testing. All of the authors discussed the results, reviewed and are responsible for the manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

Ethics approval and consent to participate
This study was approved by the Ethics Committee of the Second Affiliated Hospital of Soochow University (Ethics Approval Number: JD-LK2024171-IR01).

Consent for publication
We further confirm that the content has not been published or submitted for publication elsewhere.

Additional information
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Correspondence and requests for materials should be addressed to Y.Y. or Z.G.