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ORIGINAL RESEARCH

LC-MS/MS Method for Simultaneous Quantification of Three Oxazolidinone Antimicrobials in Human Plasma: Application to Therapeutic Drug Monitoring

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Background: Oxazolidinone antimicrobials, which are effective against multidrug-resistant gram-positive pathogens, face challenges of variable efficacy and safety owing to patient pharmacokinetic differences.

Purpose: This study aimed to establish a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to simultaneously quantify multiple oxazolidinone antimicrobials, including linezolid, tedizolid, and contezolid, for therapeutic drug monitoring (TDM) applications.

Methods: Chromatographic separation was achieved on a C18 column (100 × 2.1 mm, 3.5 μm) with gradient elution. Detection was performed via positive electrospray ionization (ESI+) in multiple reaction monitoring (MRM) mode, targeting transitions: m/z 338.14→162.8 (linezolid); m/z 371→343.1 (tedizolid) and m/z 409.15→269.14 (contezolid), with voriconazole-d3 as the internal standard.

Results: The method was validated using *Bioanalytical Method Validation (M10)*. The method demonstrated high selectivity and wide linear ranges of 50.0–15,000.0 ng/mL for linezolid and contezolid, and 25.0–7500.0 ng/mL for tedizolid, respectively, with a good linearity ($R^2 > 0.993$). The intra- and inter-day accuracy and precision were within acceptable limits. Recovery ranged from 94.4% to 104.2% in plasma, and matrix effects were negligible (CV%<3.6%). Stability experiments confirmed analyte integrity under short-term (8 h at room temperature), long-term (34 days at −80°C for linezolid; 40 days for tedizolid and contezolid), and freeze-thaw conditions. No carry-over contamination was exhibited. This method has been successfully applied to monitor the concentrations of both drugs during the transition between linezolid and contezolid therapy.

Conclusion: This validated LC-MS/MS method enables the simultaneous determination of linezolid, tedizolid, and contezolid in human plasma, rendering it promising for pharmacokinetic studies and TDM, and contributing to optimized patient care in complex therapeutic scenarios.

Keywords: oxazolidinone, linezolid, tedizolid, contezolid, LC-MS/MS

Introduction

Owing to the long-term and widespread use of antibiotics in the clinic, antimicrobial resistance has become a major global health issue, and is responsible for anti-infective treatment failure, prolonged hospitalization, and high mortality.[1] In particular, multidrug-resistant gram-positive bacteria, including *methicillin-resistant Staphylococcus aureus* (MRSA), *methicillin-resistant Staphylococcus epidermidis* (MRSE), and *vancomycin-resistant Enterococcus* (VRE) has been reported as the prominent causes of serious hospital infection in the intensive care unit (ICU).[2,3] As a recent class of synthetic antibacterial drugs active against MRSA, MRSE and VRE, etc, oxazolidinones have attracted extensive attention and showed great prospects, because of its unique antibacterial mechanism——inhibiting the initial stage of protein synthesis, and exhibiting no cross-resistance with other agents.[4,5] Currently, there are three oxazolidinone antimicrobials on the market: linezolid, tedizolid, and contezolid.[6] Linezolid was the first oxazolidinone approved by FDA in 2000 and currently employed for the treatment of community-acquired pneumonia, nosocomial pneumonia, complicated and

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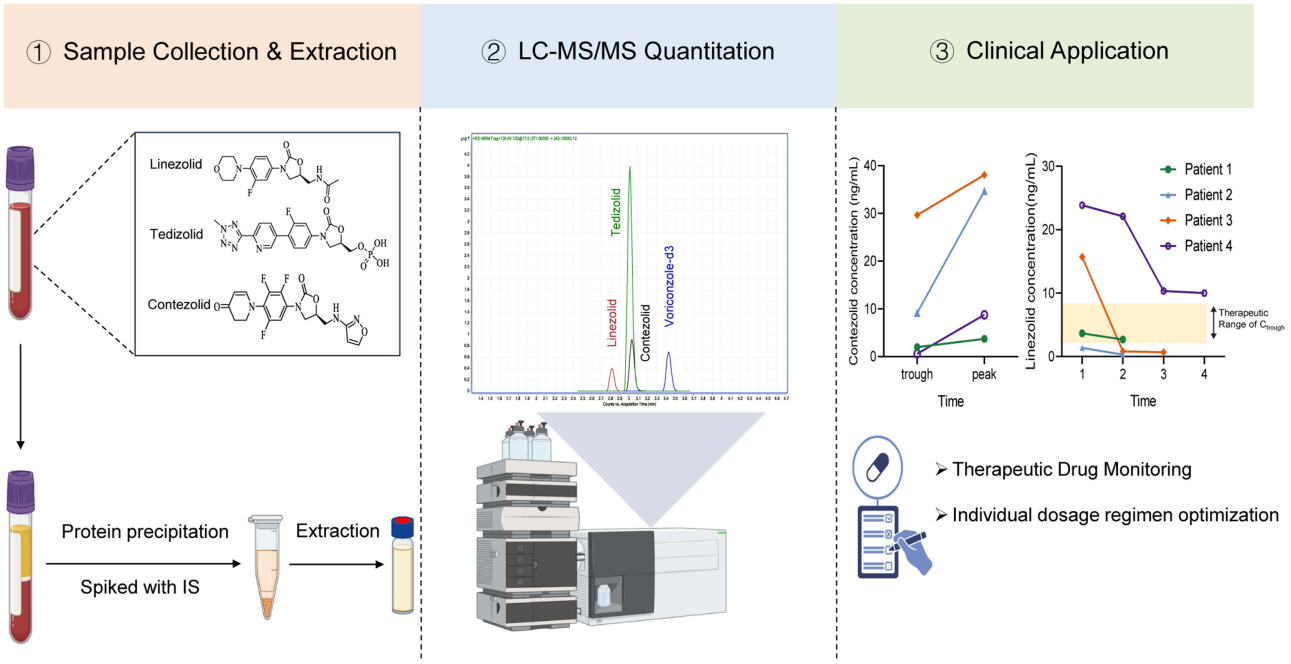
Graphical Abstract



uncomplicated skin and skin-structure infections, and infection caused by MRSA, VRE and tuberculosis.[7,8] Tedizolid is a second-generation oxazolidinone drug approved by the FDA in 2014 for the treatment of acute bacterial skin and skin structure infections (ABSSSI).[9] Contezolid is a novel oxazolidinone approved by the National Medical Products Administration of China (NMPA) in 2021 for complicated skin and soft tissue infections (cSSTIs).[10]

Based on the pharmacodynamic and pharmacokinetic profiles, the three oxazolidinones exhibited similar antibacterial spectra and mechanisms but differed in clinical applications. A meta-analysis indicated that linezolid was more effective than tedizolid in MRSA-related pneumonia, whereas tedizolid had comparable effects to linezolid in ABSSSI and a lower incidence of adverse reactions than linezolid.[11] Furthermore, clinical studies have shown that contezolid is more effective and has a lower incidence of myelosuppression than linezolid in treating cSSTIs[12] and *mycobacterium* infections.[13] Linezolid remains a preferred empirical option due to extensive evidence and broad indications, though its short half-life (4–5 h) necessitates twice-daily dosing and prolonged use risks myelosuppression or neurotoxicity; tedizolid's extended half-life (~12 h) enables once-daily dosing, enhancing outpatient compliance; contezolid demonstrates optimized metabolism (non-renal excretion) and lower hematologic toxicity, favoring long-term therapy or comorbid patients. Thus, in clinical practice, oxazolidinone-based regimens are dynamically tailored to the infection profiles, patient status, and treatment duration.

Despite the explicit mechanism, the efficacy of oxazolidinone antibiotics during routine therapy depends on their systemic exposure, which is primarily determined by the duration for which the plasma concentration exceeds the minimum inhibitory concentration (MIC) of the target pathogen (T>MIC) and the overall area under the concentration-time curve (AUC). The pharmacokinetics/ pharmacodynamics (PK/PD) target for linezolid is to achieve T>MIC≥85% and AUC/MIC>100.[14,15] Contezolid showed satisfactory efficacy against MRSA, with a cumulative fraction of response of >90% for the free drug AUC/MIC≥2.3.[16] Moreover, exceeding the therapeutic safety dose of oxazolidinones may lead to adverse events such as neurotoxicity, hematotoxicity, and thrombocytopenia.[17–19] This is predominantly attributed to the extreme inter- and intra-individual pharmacokinetic variability among patients, leading to inadequate or excessive exposure, thereby contributing to treatment failure, or drug accumulation, which is associated with toxicity.[20,21] Thus, it

is essential to provide personality antibacterial treatment to maximize therapeutic success by adjusting the dosage, which requires therapeutic drug monitoring (TDM).[22]

Although TDM of oxazolidinone is not mandatory, numerous studies have indicated that it is necessary to assess its effectiveness and safety.[11,23,24] Previous studies have focused on analyzing single oxazolidinone antibiotics in biological matrices.[25–27] Tanaka et al developed an UPLC-MS/MS method for linezolid and tedizolid in plasma, and Zhang et al reported an LC-MS/MS method for contezolid in plasma and cerebrospinal fluid. However, there is a paucity of comprehensive research on the simultaneous analysis of multiple oxazolidinones. Notably, it has been reported that myelotoxicity induced by linezolid can be corrected by switching to tedizolid and contezolid, indicating that tedizolid and contezolid have the potential to serve as alternatives to linezolid owing to their low thrombocytopenia rates.[28–30] Thus, quantifying single oxazolidinone in biological matrices limits their utility in scenarios such as therapeutic switching or combined therapy monitoring. To accurately assess drug concentrations in patients during the switch and allow for timely adjustment of drug dosage to avoid potential risks, there is an urgent need for a method capable of simultaneously quantifying multiple oxazolidinone antibacterial agents.

In this study, we developed and validated a quick and reliable liquid chromatography-tandem mass spectrometry (LC-MS /MS) method for the simultaneous determination of linezolid, tedizolid, and contezolid in the plasma of hospitalized patients, which can be applied in high-throughput assays for clinical applications and provide valuable support for rational drug use.

Materials and Method

Chemical and Reagents

Linezolid (purity: 99.0%) was purchased from Macklin Biochemical Technology Co. Ltd. (Shanghai, China). Contezolid (purity: 99.92%) was provided by MicuRx Pharmaceuticals, Inc. (Shanghai, China). Tedizolid (purity: 99.19%) and voriconazole-d3 (purity: 99.56%), an isotope-labelled internal standards (IS), were purchased from MedChemExpress LLC (Monmouth Junction, NJ, USA). HPLC-grade methanol, acetonitrile (ACN), and water as well as LC-MS grade solvents formic acid (FA) and ammonium acetate (NH_4OAc) were obtained from Thermo Fisher Scientific (Fair Lawn, NJ, USA). Dimethyl sulfoxide (DMSO; purity > 99.5%) was purchased from Beijing LABLEAD Biotech Co., Ltd. (Beijing, China).

Preparation of Calibration Standards (STDs) and Quality Control Samples (QCs)

Stock solutions of linezolid, tedizolid, contezolid, and voriconazole-d3 (1000.0 μg/mL each) were prepared in DMSO and stored at −80 °C. Working solutions of different concentrations were prepared via gradient dilution with 50% ACN (v/v) containing 0.1% FA (v/v). A series of STDs were prepared in blank plasma with concentrations of 50.0/25.0, 100.0/50.0, 250.0/125.0, 1000.0/500.0, 2500.0/1250.0, 5000.0/2500.0, 12,000.0/6000.0, 15,000.0/7500.0 ng/mL for linezolid and contezolid/ tedizolid. QC samples were prepared at the lower limit, low, medium, and high concentrations of 50.0/25.0, 150.0/75.0, 5000.0/2500.0, 10,000.0/5000.0 ng/mL, respectively. IS working solution at 250.0 ng/mL was prepared in methanol: ACN (1:1, v/v) and stored at 4 °C.

Sample Preparation

All collected blood samples were centrifuged at 3500 rpm (approximately 2123 g) for 5 min at 4 °C to obtain plasma. Fifty microliters of STDs, QCs, and plasma samples were spiked with 200 μL IS solution and homogenized for protein precipitation. The samples were centrifuged for 10 min at 14000 rpm (approximately 17968 g) at 4 °C. The samples (100 μL) were mixed with 200 μL 50% ACN (v/v) containing 0.1% FA (v/v) and transferred to autosampler vials for LC-MS/MS analysis.

LC-MS/MS Conditions

LC-MS/MS was performed using an Agilent 1260 high-performance liquid chromatograph coupled to a 6460A triple-quadrupole mass spectrometer (Agilent Technologies, Palo Alto, USA) fitted with an Agilent Jet Stream ion source and electrospray ionization in positive mode (ESI+). Chromatographic separation was performed on an Agilent Eclipse Plus C18 column (100 × 2.1 mm, 3.5 μm), using 10 mM NH_4OAc in water containing 0.1% FA (mobile phase A) and 5 mM

NH_4OAc in 90% ACN containing 0.1% FA (mobile phase B), maintained at room temperature. The autosampler injection volume was 5 μL. A gradient elution at 0.3 mL/min flow rate was performed with B (%) programmed as follows: 0.0–0.20 min, 15%; 0.20–1.50 min, 70%; 1.50–4.00 min, 98%; 4.00–6.00 min, 15%.

The following mass spectrometry parameters were applied: capillary voltage, 4000V; drying gas, 350 °C, 5 L/min; sheath gas, 350 °C, 5 L/min; nebulizer pressure, 30 psi. Ion monitoring was performed in multiple reaction monitoring (MRM) mode, with the parameters reported in Table 1.

Data acquisition and analysis were conducted using Agilent MassHunter Workstation Software (Version B.08.00).

Method Validation

The present method was fully validated in terms of linearity, selectivity, accuracy, precision, matrix effects, recovery, stability, and carryover, according to the *Bioanalytical Method Validation (M10)* of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH).

Linearity

Calibration curves for the three analytes were constructed using eight standard concentrations (50.0/25.0, 100.0/50.0, 250.0/125.0, 1000.0/500.0, 2500.0/1250.0, 5000.0/2500.0, 12,000.0/6000.0, 15,000.0/7500.0 ng/mL for linezolid and contezolid/ tedizolid). The linearity of the calibration curve was assessed by performing linear regression using the $1/X^2$ weighting factor via the peak area ratios of the target analyte and the IS. Linearity was evaluated thrice on three separate days for linezolid, tedizolid, and contezolid. The acceptance criterion for the back-calculated concentration was ± 15% of the theoretical value (± 20% for the lowest concentration).

Selectivity

The selectivity and specificity of the assay were evaluated using six drug-free blank plasma samples. Blank samples spiked with linezolid, tedizolid, and contezolid at the lower limit of quantification (LLOQ) level and blank samples spiked with only IS were processed and analyzed using this method. The absence of interfering components was considered acceptable when the signal was less than 20% of the LLOQ and less than 5% of the response to the IS.

Accuracy and Precision

Intra- and inter-day accuracy and precision were evaluated using four QC samples (lower limit, low, medium, and high concentrations) with six replicates on three separate days for linezolid, tedizolid, and contezolid, respectively. QC samples were freshly prepared daily for three days. Accuracy was measured using the mean relative error (MRE%). Precision was expressed as the relative standard deviation (RSD%). The bias was < 15.0% (< 20.0% for the LLOQ).

Matrix Effects

To measure the matrix effect, 20 μL of a mixed standard solution of the analytes at three concentration levels (LQC, MQC, and HQC) was added to 180 μL of the plasma sample and solvent solution. Matrix effects were evaluated by comparing the peak area of analytes in plasma samples reconstituted with spiked solutions to the peak area of analytes in the solvent in six replicates.

Recovery

Recovery was evaluated by comparing the peak areas of linezolid, tedizolid, and contezolid spiked before extraction to the peak areas of the analytes after extraction at the same concentration. This study analyzed low, medium, and high QC

Table 1 MRM Parameters and Retention Time (RT) for All Analytes

Analytes	Precursor/Product (m/z)	Fragmentor (V)	Collision Energy (V)	Cell Accelerator Voltage (V)	RT (min)
Linezolid	338.14/162.8	65	30	4	2.791
Tedizolid	371/343.1	130	20	4	2.946
Contezolid	409.15/269.14	80	30	4	3.011
Voriconazole-d3	353.1/129.9	130	15	4	3.424

concentration samples prepared using plasma from six different sources. Mean recovery was calculated as the average of six replicates at each individual QC concentration level, while the overall recovery was determined by averaging the mean recoveries obtained from its low, medium, and high QC concentration levels.

Stability

Stability experiments were conducted to assess the stability of the three analytes under various conditions. Short- and long-term stability tests were performed using plasma spiked at low and high QC concentrations. Short-term stability was evaluated using samples kept at room temperature at 0, 8 h intervals. Long-term stability was tested by storing the samples at −80 °C for 40 days. Autosampler stability was assessed by re-analysis after being left in autosampler for 30 h at 4 °C. Freeze-thaw stability was evaluated three freeze and thaw cycles at −80 °C. Additionally, the stability of stock solutions of the analytes and IS was also assessed. Short-term stability was evaluated using samples kept at room temperature at 15.8 h intervals, and long-term stability was evaluated at −80 °C for 204 days. A bias < 15.0% was considered stable.

Carry-Over

Carry-over was checked by injecting blank plasma samples after the highest concentration of the calibration curve. The carry-over of the analytes should be less than 20% of the peak area of the LLOQ.

Results

Methods Development

The proposed multi-analyte analysis with Agilent 1260 high-performance liquid chromatography coupled to a 6460A triple-quadrupole mass spectrometer in the positive ionization mode allowed the simultaneous determination of linezolid, tedizolid, and contezolid.

The Chromatography and MS protocols were optimized to obtain a high-throughput analytical method for clinical use. The Agilent Eclipse Plus C18 column exhibited good peak shape and separation efficiency. A mobile phase containing 10 mM NH_4OAc in water and 5 mA NH_4OAc in 90% ACN yielded the highest signal intensity. The addition of 0.1% FA was used to reduce peak trailing. Optimized mass spectrometer parameters, such as scan time (dwell time), fragmentor voltage, and collision energy, were selected to obtain the most sensitive and stable ion transitions (Table 1). Retention time (RT) for linezolid, tedizolid and contezolid were 2.79, 2.95 and 3.01 min, respectively (Figure 1).

Method Validation

Linearity

The assay was linear over a concentration range of 50.0–15,000.0 ng/mL for linezolid and contezolid, and 25.0–7500.0 ng/mL for tedizolid. The average calibration curves obtained on three different days showed good correlation coefficients ($R^2 > 0.993$) for all three analytes, and the deviations for the measured concentrations were within ± 15.0% of the nominal concentrations (Figure 2).

Selectivity and Specificity

The chromatograms of linezolid, tedizolid, and contezolid and the corresponding IS in the plasma samples are shown in Figure 2. No interference was observed in the analyte retention times. For the blank plasma samples, the signals were below 20% of the LLOQ for the three analytes, thus ensuring a high selectivity and specificity (Figure 3).

Accuracy and Precision

The intra- and inter-day accuracy and precision, expressed as mean relative error (MRE%) and relative standard deviation (RSD%), respectively, for the LLOQ, LQC, MQC, and HQC levels of the three analytes are shown in Table 2. The intra- and inter-day accuracy for the three analytes ranged between 93.8%-112.4% and 96.9%-108.5%, while the intra- and inter-day precision were between 0.5%-6.7% and 1.2%-6.8%, within the 15% limit requested by the guideline.

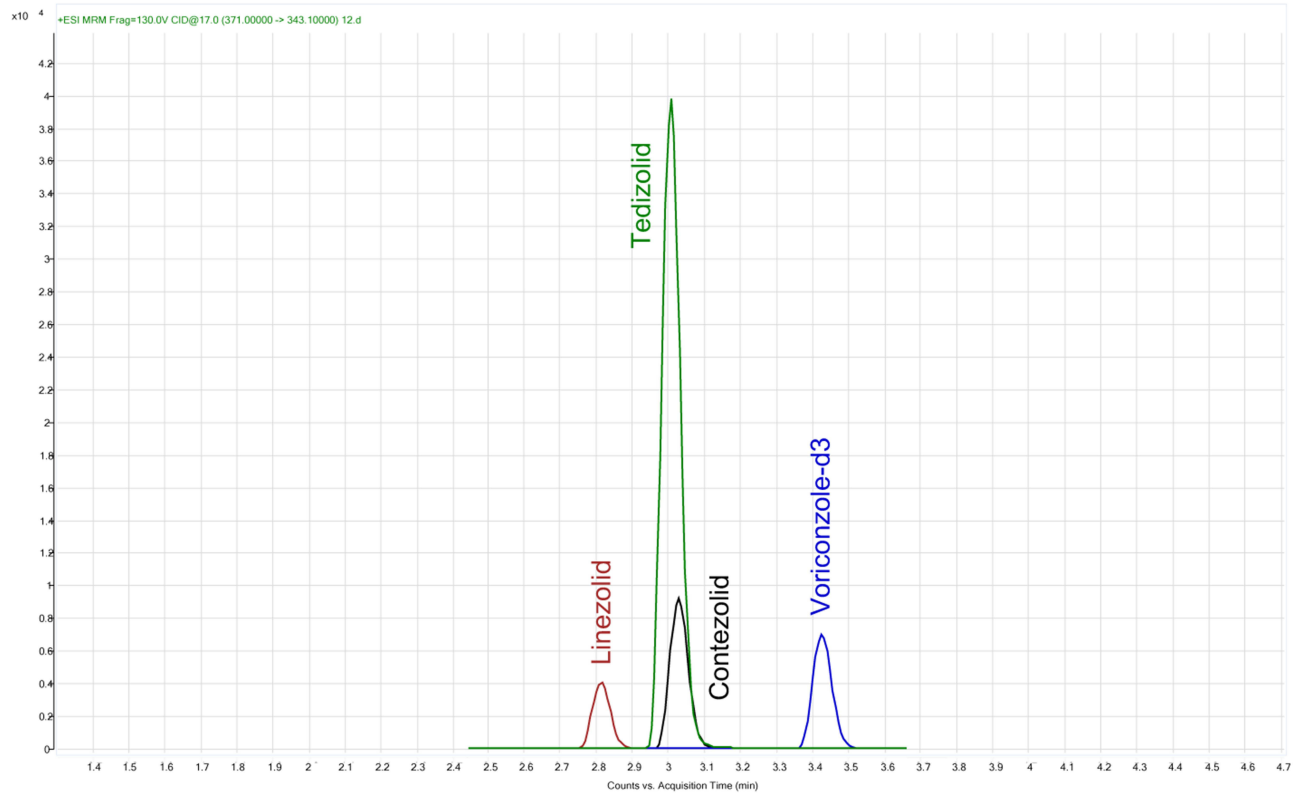


Figure 1 Representative chromatogram of linezolid, tedizolid, contezolid and the corresponding IS in human plasma. Retention time (RT) for linezolid, tedizolid, contezolid and IS were 2.79, 2.95, 3.01 and 3.424 min, respectively.

Matrix Effects

Matrix effects serve as indicators of the accuracy of the extraction procedure.[31] The mean matrix effect at LQC, MQC and HQC for the three analytes was between 0.96 and 1.02 in normal plasma, with the coefficient of variation (CV%) ranged from 1.4% to 3.4%, which was inside the ranges of acceptance. The matrix effect of the analytes at the LQC and HQC in hemolysis and hyperlipidemia was between 0.95 and 1.14, with CV% ranging from 0.1% to 3.6% (Table 3).

Recovery

The recovery of all three analytes ranged from 94.4% to 104.2%. The bias of the calculated values compared to the added theoretical concentrations was within 6.6% (Table 4).

Stability

The stability assays for the three compounds at the low and high QC levels are summarized in Table 5. The three analytes remained stable for 8 h at room temperature and 30 h in an autosampler at 4 °C. Tedizolid and contezolid were stable for 40 days at −80 °C, whereas degradation was observed for linezolid. We then evaluated the long-term stability of linezolid for 34 days at −80 °C. All the analytes were stable for at least three freeze-thaw cycles when stored at −80 °C. The stability assays for the stock solutions are summarized in Table 6. The three analytes and IS remained stable for 15.8 h at room temperature and stable for 204 days at −80 °C.

Carry-Over

Carry-over was assessed as null, as there was no signal above the detection limit of the blank plasma in the retention time of the analytes after injection of the HQC samples.

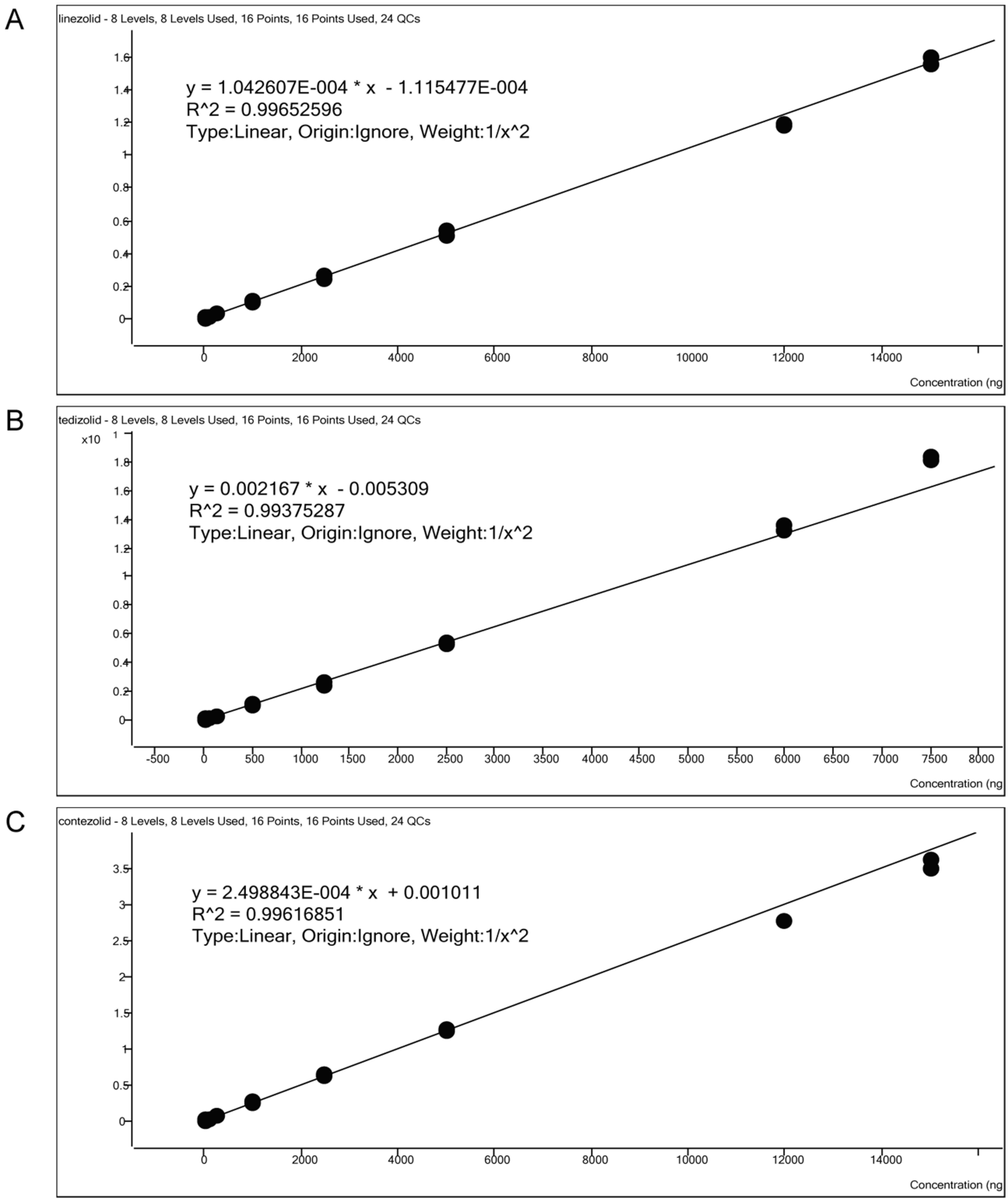


Figure 2 Linear calibration curves for linezolid (**A**), tedizolid (**B**) and contezolid (**C**). The graphs depict the relationship between concentration (ng/mL) and relative response for each analyte. They highlight the assay's linearity over concentration ranges of 50.0–15,000.0 ng/mL for linezolid and contezolid, and 25.0–7500.0 ng/mL for tedizolid.

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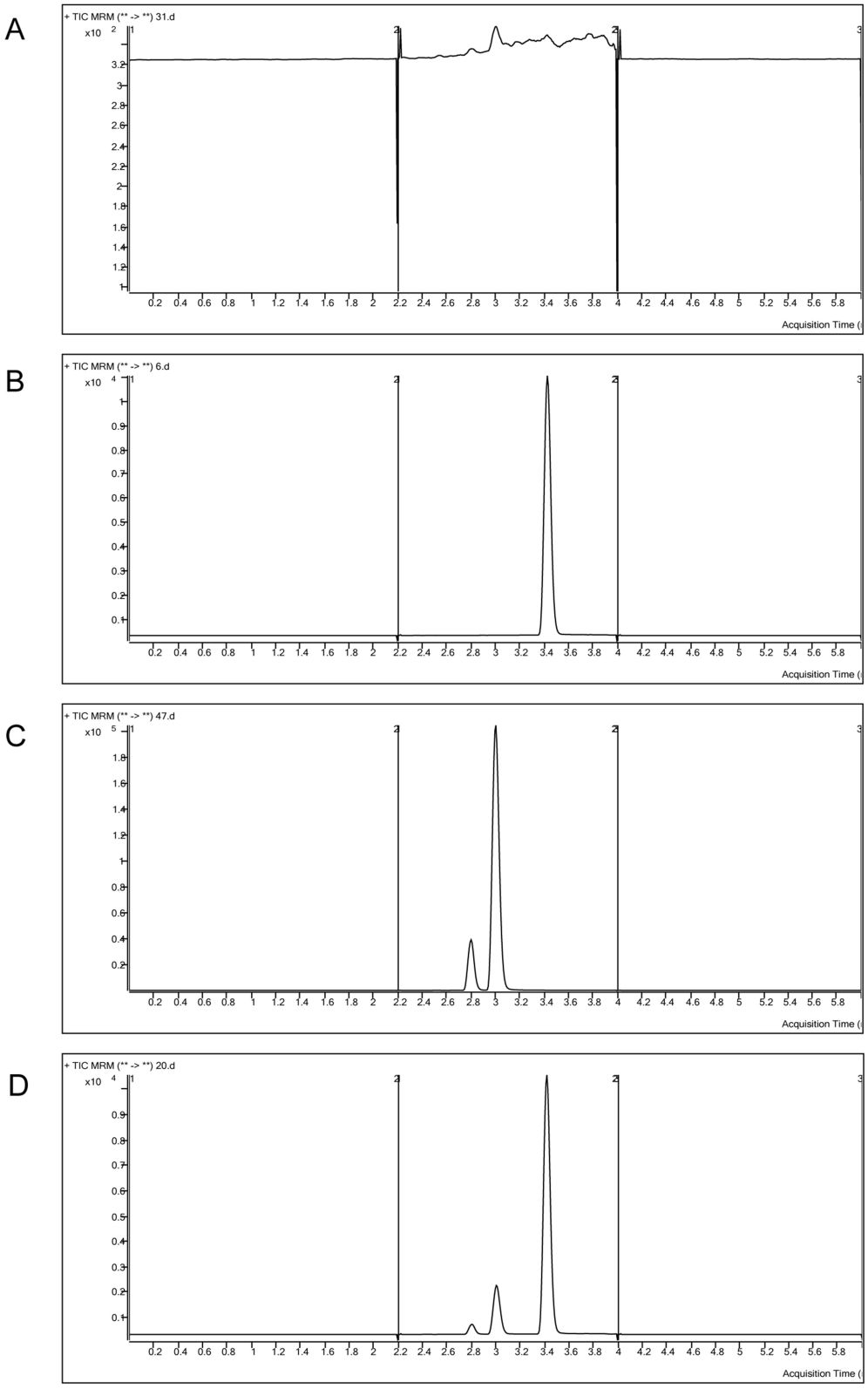

Figure 3 Selectivity chromatograms of the target compound and interferents. (**A**) Double blank. (**B**) IS only. (**C**) Linezolid, tedizolid and contezolid. (**D**) LLOQ of Linezolid, tedizolid, contezolid and IS.

Table 2 Intra- and Inter-Day Accuracy and Precision for Linezolid, Tedizolid and Contezolid

Analytes	Concentration (ng/mL)	Accuracy		Precision	
		Intra-Day (%)	Inter-Day (%)	Intra-Day (%)	Inter-Day (%)
Linezolid	50.0	108.71	107.62	5.35	4.96
		112.33		6.70	
		101.82		5.32	
	150.0	111.22	104.78	4.34	6.79
		105.97		3.41	
		97.14		2.36	
	5000.0	101.31	100.08	2.05	1.58
		100.62		2.51	
		98.30		0.51	
	10,000.0	102.01	100.20	2.22	2.13
		100.73		1.85	
		97.85		1.30	
Tedizolid	25.0	106.28	106.83	4.00	2.17
		109.38		3.89	
		104.84		2.70	
	75.0	105.59	102.05	4.08	3.95
		102.90		2.87	
		97.66		2.33	
	2500.0	103.77	102.33	3.97	1.23
		101.47		1.47	
		101.74		2.02	
	5000.0	109.25	108.49	3.76	1.72
		109.86		1.38	
		106.36		1.09	
Contezolid	50.0	98.35	99.01	5.43	3.31
		102.57		4.20	
		96.11		3.87	
	150.0	111.00	106.13	2.57	5.86
		108.27		2.98	
		99.12		1.69	
	5000.0	101.88	100.35	1.85	1.66
		100.61		2.41	
		98.58		1.27	
	10,000.0	98.04	96.94	2.09	2.78
		98.90		1.31	
		93.87		1.59	

Table 3 Matrix Effects of Linezolid, Tedizolid and Contezolid

Analytes	Concentration (ng/mL)	Plasma		Hemolytic Plasma		Hyperlipidemic Plasma	
		MF	CV (%)	MF	CV (%)	MF	CV (%)
Linezolid	150.0	1.0158	2.50	1.1104	3.34	1.1397	3.57
	5000.0	0.9863	2.96	——	——	——	——
	10,000.0	0.9725	3.00	0.9942	0.26	1.0405	0.19
Tedizolid	75.0	1.0183	2.38	1.0727	0.80	1.0724	0.53
	2500.0	0.9975	3.40	——	——	——	——
	5000.0	1.0069	1.49	1.1019	0.39	1.1340	0.20

(*Continued*)

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Table 3 (Continued).

Analytes	Concentration (ng/mL)	Plasma		Hemolytic Plasma		Hyperlipidemic Plasma	
		MF	CV (%)	MF	CV (%)	MF	CV (%)
Contezolid	150.0	0.9703	2.81	1.0930	2.57	1.1289	2.91
	5000.0	0.9655	2.11	——	——	——	——
	10,000.0	0.9655	1.51	0.9595	0.35	1.0019	0.62

Abbreviation: MF, matrix factor.

Table 4 Recovery of Linezolid, Tedizolid and Contezolid

Analytes	Concentration (ng/mL)	Average Recovery		Overall Recovery	
		Recovery (%)	CV (%)	Recovery (%)	CV (%)
Linezolid	150.0	104.14	1.89	98.18	5.64
	5000.0	94.96	1.83		
	10,000.0	94.53	2.98		
Tedizolid	75.0	101.46	4.78	96.24	6.31
	2500.0	94.50	4.02		
	5000.0	92.77	6.53		
Contezolid	150.0	103.43	3.22	97.71	5.09
	5000.0	95.23	1.61		
	10,000.0	94.47	3.60		

Clinical Application

The validated LC-MS/MS method was applied to TDM of linezolid, tedizolid, and contezolid in plasma samples from hospitalized patients. The quantified drug concentrations in most patients were within the validated range, except for a few samples that exceeded the linear range and required dilution. Here, we demonstrated several samples containing contezolid and linezolid simultaneously from four patients aged 78–98 years, due to the therapeutic switch from linezolid to contezolid. From the perspective of efficacy and safety, 2–8 μg/mL is recommended as the therapeutic range for linezolid trough concentration.[11,32,33] As shown in Table 7, the plasma concentration of linezolid in patient 3 was 15.720 μg/mL, which decreased to 0.820 μg/mL after 96 h and was still not completely eliminated. After patient 4 was switched from linezolid to contezolid, the initial plasma concentration of linezolid was approximately 22 μg/mL and remained above 10 μg/mL after 48 h, still exceeding the safety threshold. The dosage of contezolid was halved in super-elderly patients (> 96 years: 400 mg/q12 vs 800 mg/q12 in 78 years). Higher dosages of contezolid (800 mg/q12) correlated with higher trough concentrations (11.764 μg/mL), whereas super-elderly patients (400 mg/q12h) exhibited variable trough concentrations (0.580–9.161 μg/mL) and peak concentrations (3.179–34.698 μg/mL).

Discussion

In this study, a new LC-MS/MS method was developed to simultaneously detect oxazolidinone antibacterial agents including linezolid, tedizolid, and contezolid in human plasma samples. Protein precipitation with acetonitrile: methanol (1: 1) was chosen as the sample preparation procedure owing to its distinct advantages over solid-phase extraction and liquid-liquid extraction—particularly its high efficiency, which is well-suited for the rapid processing of large-scale samples. Method validation further demonstrated that the protein precipitation method achieved high recovery rates and no significant matrix effect was observed. Implementation of the method in routine use is further supported by the small amount of plasma sample (50 μL) and short chromatographic time (6 min). Thus, it is ideal to process a large number of samples in a short time. The addition of ammonium acetate and 0.1% formic acid to the mobile phase improved analyte response. This method has excellent linearity, with a calibration range of 50.0–15,000.0 ng/mL for linezolid and

Table 5 Stability Assays for Linezolid, Tedizolid and Contezolid

Analytes	Concentration (ng/mL)	Short-Term Stability		Long-Term Stability		Autosampler Stability		Freeze-Thaw Stability	
		Accuracy (%)	Precision (CV%)	Accuracy (%)	Precision (CV%)	Accuracy (%)	Precision (C%)	Accuracy (%)	Precision (CV%)
Linezolid	150.0	106.43	4.83	102.74	3.20	108.29	3.86	100.03	2.71
	10,000.0	106.10	0.73	102.87	0.15	98.29	3.13	106.83	0.25
Tedizolid	75.0	104.84	0.99	87.71	3.32	102.74	1.51	99.81	0.46
	5000.0	112.78	0.22	91.45	4.35	105.45	2.72	112.96	0.74
Contezolid	150.0	109.18	2.89	92.44	1.03	110.78	1.79	105.28	1.82
	10,000.0	101.54	0.43	88.35	2.81	95.61	2.91	102.24	0.13

Notes: Storage stability of low and high QC of each analyte after 8 h at room temperature (short-term stability), 30 h at 4 °C in the autosampler (autosampler stability), and three freeze-thaw cycles at −80 °C (freeze-thaw stability). Long-term stability assay for tedizolid and contezolid were 40 days at −80 °C, while 34 days at −80 °C for linezolid.

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Table 6 Stability Assays for Stock Solution of Linezolid, Tedizolid, Contezolid and Voriconazole-d3

Analytes	Concentration (μg/mL)	Short-Term stability		Long-Term stability	
		Accuracy (%)	Precision (CV%)	Accuracy (%)	Precision (CV%)
Linezolid	1000.0	100.92	1.55	90.99	0.37
Tedizolid	1000.0	99.94	2.70	91.38	0.51
Contezolid	1000.0	98.16	1.39	90.87	0.85
Voriconazole-d3	1000.0	100.23	1.30	98.41	1.68

Notes: Storage stability of stock solutions of each analyte and IS after 15.8 h at room temperature (short-term stability), and for 204 days at −80 °C (long-term stability).

Table 7 Patient Characteristics and Plasma Concentrations of Contezolid and Linezolid in Patients

Patient	Gender	Age (Year)	Dosage of CZD	Time Point	Plasma Concentration (μg/mL)	
					Contezolid	Linezolid
1	Male	96	400 mg/q12	Prior to administration of CZD	1.999	3.674
				2.5 h after administration of CZD	3.719	2.685
2	Male	97	400 mg/q12	Prior to administration of CZD	9.161	1.35
				2.5 h after administration of CZD	34.698	0.32
3	Male	78	800 mg/q12	Prior to administration of CZD	11.764	15.720
				Prior to administration of CZD*	29.659	0.820
				2.5 h after administration of CZD	38.075	0.690
4	Male	98	400 mg/q12	6.5 h after administration of CZD	10.156	23.860
				8.5 h after administration of CZD	7.067	22.117
				Prior to administration of CZD#	0.580	10.347
				2.5 h after administration of CZD	8.749	10.005

Notes: Contezolid was administered orally. Blood collection time points prior to administration of contezolid was defined as trough concentration (C_{trough}). Blood collection time points 2.5 h after administration of contezolid was defined as peak concentration (C_{peak}). CZD: contezolid. *96 hours after the first TDM. #48 hours after the first TDM.

contezolid, and 25.0–7500.0 ng/mL for tedizolid, with correlation coefficient (R^2>0.993). The distinct linear range is attributed to the lower recommended dose and frequency of tedizolid compared to linezolid and contezolid (200 mg/d vs 600 mg/12 h). Compared to previously reported method to detect linezolid or tedizolid, our method was more sensitive with a lower LLOQ (50.0 ng/mL for linezolid and 25.0 ng/mL for tedizolid).[24,34] Although the stability of linezolid at −80 °C for 34 days was demonstrated, the degradation was observed during the 40-day assessment. Thus, linezolid-containing plasma samples should be analyzed within 34 days of collection (at −80 °C) to ensure analyte integrity. In clinical practice, all plasma samples for TDM were analyzed within 48 hours after collection.

As the mechanisms of action of linezolid, tedizolid, and contezolid are similar, the pharmacokinetics of the three drugs are different. Linezolid, tedizolid, and contezolid undergo distinct hepatic metabolic pathways, respectively mediated by microsome-mediated morpholine ring oxidation,[35] sulfotransferases (SULTs)[36] and flavin-containing monooxygenase-5 (FMO5).[37] Approximately 30% of linezolid is a prototype and 50% of its metabolites are excreted in the urine,[38] whereas tedizolid is mainly excreted by the liver in feces[39] and approximately 76% of contezolid is metabolized and eliminated via the urinary route.[40] The elimination half-lives of linezolid, tedizolid, and contezolid are 4–6 h, 12 h and 2–3 h, respectively, which influence the dosage and dosing frequency.[10,41,42] Remarkably, there is a limitation in the treatment duration of oxazolidinones in the clinical setting. The recommended duration for linezolid and tedizolid is generally limited to 28 days and 6 days, respectively, as prolonged treatment may increase the risk of hematologic and

neurologic toxicity.[19] However, extended courses of oxazolidinones therapy in real-life are not rare in the treatment of severe or complicated infections, such as drug-resistant tuberculosis. Given the distinct metabolism and half-lives of the three oxazolidinones, therapeutic switching is prone to risks like residual pre-switch drug accumulation and overlapping post-switch drug concentrations, which single-analyte monitoring cannot solve. This simultaneous quantification enables tracking of both pre- and post-switch drug dynamics during transition, directly preventing toxicity from pre-drug buildup and guiding precise dose adjustment of the new agent. Thus, TDM is strongly recommended for patients requiring prolonged treatment durations.

To optimize the individual dosage regimens of oxazolidinone, we applied this method to a clinical setting by measuring samples from patients admitted to our hospital. Patient analysis showed that significantly prolonged elimination (decreased to approximately 1 μg/mL: 96 h vs 24 h[43]) of linezolid was observed in elderly patients, resulting in the accumulation of linezolid at concentrations far exceeding the safety threshold, even after switching to contezolid. Although the dosage of contezolid was adjusted according to patient status (age), the interindividual variability remained significant in contezolid exposure (C_{trough}: 0.580–9.161 μg/mL, C_{peak}: 3.719–34.698 μg/mL, at a dose of 400 mg/q12), which may be attributed to age-related progressive hepatorenal dysfunction in elderly patients. However, Cattaneo et al revealed that nearly 70% of patients aged > 80 years had linezolid trough concentrations > 8 μg/mL, with an increased risk of adverse effects.[32] Wu et al showed that patients with moderate hepatic impairment had a lower maximum concentration (C_{max}) of contezolid and a longer time to C_{max} (T_{max}) than healthy controls.[44] Moreover, because tedizolid is primarily eliminated through the liver, the FDA Adverse Event Reporting System has found that the incidence of hepatic failure reports with tedizolid is higher than linezolid.[45] Thus, the simultaneous determination of oxazolidinones can serve as an indispensable tool for maximizing antimicrobial efficacy while minimizing toxicity, particularly in populations at risk of pharmacokinetic alterations.

Our study successfully demonstrated the method's utility in a small cohort of four elderly patients undergoing a switch from linezolid to contezolid. Notably, we observed significantly prolonged linezolid elimination and substantial pharmacokinetic (PK) variability in this group, aligning with the findings of the multicenter study, which identified advanced age, renal dysfunction, and hepatic impairment as key drivers of PK unpredictability.[46] This concordance underscores the vital role of TDM in these high-risk populations. The ability to seamlessly measure both the declining concentration of the previous drug (linezolid) and the rising concentration of the new drug (contezolid) with a single, rapid assay is invaluable. It prevents critical gaps in antimicrobial coverage and mitigates the risk of additive toxicity during the transition period. These findings highlight the immediate clinical value of our method; to further extend its applicability for precise TDM across diverse settings, future validation in other stratified populations will be essential. Future work will focus on applying the method to elderly and pediatric patients, as well as other special populations. Based on accumulated clinical data, in-depth population pharmacokinetic studies will be conducted to guide precision medicine in the clinical use of oxazolidinones.

Conclusion

In this study, we developed and validated a novel LC-MS/MS method for the simultaneous quantification of three oxazolidinones (linezolid, tedizolid, and contezolid) in the human plasma. The optimized method demonstrated several advantages, including simplicity, rapid analysis time, high sensitivity, and excellent specificity. Furthermore, the successful application of TDM has revealed its clinical utility in optimizing antimicrobial therapies.

Institutional Review Board Statement

This study was approved by the Medical Ethics Committee of Chinese PLA General Hospital (Approval No. S2021-609-01), which waived the requirement for informed consent due to the retrospective analysis of anonymized TDM data. All patient data were handled with strict confidentiality to ensure privacy protection.

Abbreviations

ABSSSI, acute bacterial skin and skin structure infections; cSSTIs, complicated skin and soft tissue infections; FMO-5, flavin-containing monooxygenase-5; ICH, International Council for Harmonization; ICU, intensive care unit; LC-MS /MS, liquid chromatography-tandem mass spectrometry; IS, internal standard; LLOQ, lower limit of quantification;

MRM, multiple reaction monitoring; MRSA, *methicillin-resistant Staphylococcus aureus*; MRSE, *methicillin-resistant Staphylococcus epidermidis*; NMPA, National Medical Products Administration of China; PK, pharmacokinetic; QC, quality control; TDM, therapeutic drug monitoring; VRE, *vancomycin-resistant Enterococcus.*

Data Available Statement

The authors confirm that data supporting the findings of this study are available within the article.

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Author Contributions

Na Zhang: Methodology, Validation, Formal analysis, Investigation; Nan Bai: Methodology, Validation, Formal analysis, Investigation; Ying Wang: Writing - Original Draft, Review & Editing, Visualization; Beibei Liang: Investigation, Writing-Review & Editing; Yun Cai: Conceptualization, Writing-Review & Editing, Supervision, Project administration, Funding acquisition. All authors gave final approval of the version to be published and agreed to be accountable for all aspects of the work.

Disclosure

The authors declare no competing interests in this work.

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