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RESEARCH ARTICLE

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UHPLC–MS/MS method for simultaneous quantification of doripenem, meropenem, ciprofloxacin, levofloxacin, pazufloxacin, linezolid, and tedizolid in filtrate during continuous renal replacement therapy

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Abstract

Background: Since severe infections frequently cause acute kidney injury (AKI), continuous renal replacement therapy (CRRT) is often initiated for regulation of inflammatory mediators and renal support. Thus, it is necessary to decide the antibiotic dosage considering the CRRT clearance in addition to residual renal function. Some of the hemofilters used in CRRT are known to adsorb antibiotics, and clearance of antibiotics may differ depending on the adsorptive characteristics of hemofilters. Although assay systems for blood and CRRT filtrate concentrations are required, no method for measuring antibiotics concentrations in filtrate has been reported. We developed a UHPLC–MS/MS method for simultaneous quantification of antibiotics commonly used in ICU, comprising carbapenems [doripenem (DRPM) and meropenem (MEPM)], quinolones [ciprofloxacin (CPFX), levofloxacin (LVFX) and pazufloxacin (PZFX)] and anti-MRSA agents [linezolid (LZD), and tedizolid (TZD)] in CRRT filtrate samples.

Methods: Filtrate samples were pretreated by protein precipitation. The analytes were separated with an ACQUITY UHPLC CSH C18 column under a gradient mobile phase consisting of water and acetonitrile containing 0.1% formic acid and 2 mM ammonium formate.

Results: The method showed good linearity over wide ranges. Within-batch and batch-to-batch accuracy and precision for each drug fulfilled the criteria of the US Food and Drug Administration guidance. The recovery rate was more than 87.20%. Matrix effect ranged from 99.57% to 115.60%. Recovery rate and matrix effect did not differ remarkably between quality control samples at different concentrations.

Conclusion: This is the first report of a simultaneous quantification method of multiple antibiotics in filtrate of CRRT circuit.

KEYWORDS
antibiotics, continuous renal replacement therapy, filtrate, hemofilter, intensive care unit, ultra-high-performance liquid chromatography

1 | INTRODUCTION

For the treatment of severe infections in intensive care unit (ICU), administration of broad-spectrum antibiotics selected based on the assumed focus of infection and causative bacteria is recommended.[1] Sepsis often causes acute kidney injury (AKI) due to factors such as shock, decreased circulating blood flow due to systemic inflammatory response syndrome, inflammation in the kidney, and tubular damage.[2] According to a cohort study of 1177 patients with sepsis in ICU conducted at 198 institutions in 24 European countries, 51% of the patients developed AKI with mortality of 41%,[3] indicating that septic AKI in the ICU is one of the important complications. Continuous renal replacement therapy (CRRT) is often initiated in patients with septic AKI for the regulation of inflammatory mediators and renal support. However, since drugs smaller than the membrane pores of hemofilters are removed by filtration and diffusion, drugs having properties such as small molecular weight, small volume of distribution, hydrophilicity, and low protein binding rate are susceptible to removal.[4] Thus, it is necessary to decide the antibiotic dosage considering the CRRT clearance in addition to the residual renal function.

Hemofilters used in CRRT are classified into two types based on their cytokine adsorption properties: non-adsorptive membranes such as asymmetric cellulose triacetate (ATA) and polyethersulfone (PES) membranes, and adsorptive membranes such as polymethyl methacrylate (PMMA) and AN69ST membranes.[5,6] These hemofilters have been reported to adsorb some antimicrobials. Adsorptive membranes adsorb some drugs by their hydrophobic and ionic bonding properties.[7–11] On the contrary, non-adsorptive membranes also adsorb some drugs, although the mode of binding remains unclear.[12–15] In vitro studies showed 90% adsorption of gentamicin on polyacrylonitrile copolymer (PAN) membranes,[14] 90% adsorption of tigecycline on polysulfone (PS) membranes,[13] 61.4% adsorption of teicoplanin on PS membranes, 75.6% on PMMA membranes,[9] and 22% adsorption of linezolid on PMMA membranes.[7] In clinical practice, blood teicoplanin concentration was lower than expected when administered under CRRT using PMMA membranes, indicating adsorption of teicoplanin on PMMA membranes.[16] These suggest the need to adjust the dose according to the type of membrane. However, because the dosage of antibiotics in patients under CRRT is generally set considering filtration and diffusion only, without taking into account the differences in adsorption properties between hemofilters,[17] some combinations of hemofilters and antibiotics may fail to achieve pharmacokinetic and pharmacodynamics goals, resulting in treatment failure or emergence of resistant bacteria.

In addition, only in vitro or ex vivo studies on the adsorption of antibiotics to hemofilter have been reported, and those findings have not been sufficiently verified in vivo. Given the difficulties to develop an experimental system that simulates human blood and the pathological condition of AKI, the clinical significance of the in vitro and ex vivo data remains unclear. Therefore, it is very important to evaluate the adsorption of antibiotics on hemofilter in vivo. However, to examine the adsorptive properties of hemofilters in vivo, it is necessary to measure the drug concentrations in blood before and after passing through the hemofilter and drug concentration in the filtrate through the hemofilter.[18] Thus, a measurement system that can measure both blood and filtrate concentrations is required. Although there are many methods that measure the concentrations of antibiotics in blood using high-performance liquid chromatography and ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC–MS/MS),[19–23] no method that measures the antibiotic concentrations in filtrate has been documented.

With this background, we aimed to establish a system for evaluating the antibiotic adsorption properties of various hemofilters by developing a UHPLC–MS/MS assay for CRRT filtrate, which can simultaneously quantify carbapenems [doripenem (DRPM) and meropenem (MEPM)], quinolones [ciprofloxacin (CPFX), levofloxacin (LVFX), and pazufloxacin (PZFX)] and anti-MRSA agents [linezolid (LZD) and tedizolid (TZD)] that are commonly used in ICU patients.

2 | MATERIALS AND METHODS

2.1 | Materials

Chemicals are shown with purity in parentheses. Standard substances of DRPM (98%), MEPM (98%), LZD (98%), CPFX (98%), and LVFX (98%) were purchased from Sigma-Aldrich (St. Louis), and those of TZD (98%) and PZFX (98%) from Toronto Research Chemicals. For the isotopically labeled internal standard (IS), MEPM-d_6 (97%), LZD-d_3 (98%), TZD-d_3 (98%) CPFX-d_8 (98%), and LVFX-d_8 (98%) were purchased from Toronto Research Chemicals; DRPM-d_4 (98%) from Alsachim; and PZFX-d_4 (98%) from Santa Cruz Biotechnology. Acetonitrile, methanol (MeOH), ultrapure water (H_2O), 2-propanol, ammonium formate, formic acid (all chromatographical grade), and dimethyl sulfoxide (analytical grade) were purchased from Wako Pure Chemical Ind. Ltd.

2.2 | CRRT modalities and hemofilters

Continuous renal replacement therapy was performed using a roller pump (TR-55X; Toray) with one of the following dialyzers: ATA filters with surface area of 2.1 m^2 (AUT-21eco; Nipro), PES filters with surface area of 2.1 m^2 (PUT-21eco; Nipro), PMMA filters with surface area of 1.8 m^2 (CH-1.8 W; Toray) and AN69ST filters with surface area of 1.5 m^2 (Sepxiris150; Baxter). The initial blood flow rate was set at 100 ml/min, and CRRT dose was set at 600 ml/h (continuous venovenous hemofiltration), which was adjusted according to clinical need. The post-dilution technique was applied, and fluid to substitute ultrafiltrate production was reinfused after hemofiltration.

2.3 | Patients and sample collection

Patients who were treated with intravenous DRPM, MEPM, LZD, TZD, CPFX, LVFX, or PZFX in ICU at Oita University Hospital were recruited. Filtrate samples were collected from a filtrate drainage line into tubes containing ethylenediaminetetraacetic acid. At more than 3 days after starting one or more of the above antibiotics, filtrate sample was collected at 1 h (C_{max}) after infusion of the antibiotics. The collected filtrate sample was stored at −40°C until measurement. The study was conducted in accordance with the ethical standards of our institute and with the Helsinki Declaration of 1975, as revised in 2013. The protocol for this study was approved by the Ethics Committee of Oita University Faculty of Medicine (review reference number: 1557) before the study was started. Written informed consent was obtained from the patients participating in this study or their legally authorized representatives.

2.4 | Preparation of stock and working solutions

The concentrations and solvents used to prepare stock solutions of the antibiotics were determined by referring to previous reports.[19–25] Polypropylene tubes were used in this study, because our previous study found no adsorption of any drug to polypropylene tubes.[26] The stock solutions of standards and IS were prepared at the following concentrations using the solvent indicated: DRPM (1 mg/ml in 25% MeOH), DPRM-d_4 (0.1 mg/ml in 25% MeOH), MEPM (1 mg/ml in 25% MeOH), MEPM-d_6 (0.1 mg/ml in 25% MeOH), LZD (2.5 mg/ml in 25% MeOH), LZD-d_3 (0.1 mg/ml in 25% MeOH), TZD (0.2 mg/ml in dimethyl sulfoxide), TZD-d_3 (0.04 mg/ml in MeOH), CPFX (1 mg/ml in 25% MeOH + 20 mM acetic acid), CPFX-d_8 (0.25 mg/ml in 50% MeOH + 20 mM acetic acid), LVFX (1 mg/ml in 20% MeOH), LVFX-d_8 (0.1 mg/ml in 20% MeOH), PZFX [1 mg/ml in MeOH + HCl (99.5:0.5, v/v)], and PZFX-d_4 [0.1 mg/ml in MeOH + HCl (99.5:0.5, v/v)]. These stock solutions were stored at −40°C.

The working standard solutions were prepared by diluting the respective stock solutions with 50% MeOH to the following concentrations: DRPM 100 μg/ml, MEPM 250 μg/ml, LZD 100 μg/ml, TZD 10 μg/ml, CPFX 50 μg/ml, LVFX 100 μg/ml, and PZFX 100 μg/ml. These concentrations corresponded to the upper limit of the calibration ranges. The working IS solutions were prepared by diluting the respective stock solutions with 50% MeOH to the following concentrations: DRPM-d_4 10 μg/ml, MEPM-d_6 5 μg/ml, LZD-d_3 10 μg/ml, TZD-d_3 1 μg/ml, CPFX-d_8 1 μg/ml, LVFX-d_8 2 μg/ml, and PZFX-d_4 4 μg/ml. Calibrators were prepared by diluting the working solutions (upper limit of calibration range) 500-, 200-, 100-, 50-, 20-, 5-, and 2-fold using 50% MeOH.

Stock solutions and working solutions for quality controls (QCs) had the same concentrations as those for the calibrators, but were prepared separately. Lower limit of quantification (LLOQ), low QC (QC A), middle QC (QC B), and high QC (QC C) samples were prepared by diluting the working solutions for QC 20/10000, 60/10000, 600/10000, and 7500/10000, respectively, using 50% MeOH.

2.5 | Sample preparation

Filtrate samples were pretreated by protein precipitation with 50% MeOH and acetonitrile. Blank filtrate was prepared using plasma obtained from healthy volunteers. Plasma sample was centrifuged in an Amicon® Ultra-15 centrifugal filter device (Merk Millipore Ltd.) at 20,600 × g at 4°C until almost all the plasma was filtered, and the supernatant was collected. For calibrator and QC samples, 50 μl of blank filtrate was transferred to a 2-ml polypropylene tube, and 50 μl of each calibrator or QC sample in 50% MeOH, 25 μl of IS working solution in 50% MeOH, and 100 μl of acetonitrile were added in that order. For patient filtrate and blank filtrate samples, 50 μl of filtrate sample, 25 μl of IS solution in 50% MeOH, 100 μl of acetonitrile, and 50 μl of 50% methanol were added in that order into a polypropylene tube. The mixtures were vortexed for 1 min, and centrifuged at 20,600 × g at 4°C for 10 min. The supernatants were collected and transferred to a 96-well collection plate (Waters Corp.). The sample plate was sealed with a sealing cap (Waters Corp.) and kept at 4°C until assay. Twenty microliters of sample was injected into the UHPLC.

2.6 | Liquid chromatography condition and instrumental analysis parameter

The chromatographic conditions of the instrumental parameter of UHPLC–MS/MS system were set according to the report by Kai et al.,[26] In brief, an Acquity UPLC® I-Class System (Waters Corp.) with a triple-stage quadrupole mass spectrometer (Xevo TQ-D) was used with an Acquity CSH C18 column (1.7 μm, 2.1 × 50 mm) and an Acquity CSH C18 Van-Guard pre-column (1.7 μm, 2.1 × 5 mm) (both Waters Corp.). The column temperature was 40°C, and sample temperature was 4°C. The mobile phase contained 0.1% formic acid and 2 mM ammonium formate in ultrapure water-acetonitrile (98:2, v/v for solution A and 2/98, v/v for solution B). The gradient profile was 100% A 0.5 min, changing linearly to 100% B 0.5–1.5 min, holding 1.5 min, returning to 100% A, and holding 4 min. The flow rate was 0.4 ml/min. The total analysis time was 7 min.

The ionization parameters were as follows: cone voltage 28 V, electrospray voltage 2.5 kV, source temperature 150°C, cone gas flow (N_2) 50 L/h, desolvation gas flow (N_2) 800 L/h, and desolvation temperature 600°C. The mass spectrometer was tuned automatically to each drug and IS, using the MassLynx V4.1 system software package (Waters Corp.) by the IntelliStart standard optimization procedures. Multiple reaction monitoring (MRM) analysis was performed using argon as collision gas, and the MS/MS transitions monitored in the positive ion mode are listed in Table 1. The dwell time for each transition was 8 ms.

2.7 | Full validation

The assay was fully validated according to the guidelines for bioanalytical method validation of the US Food and Drug Administration (FDA).[27]

TABLE 1 MS transition data of DRPM, MEPM, CPFX, LVFX, PZFX, LZD, and TZD.

Compound	Parent ion (m/z)	Cone voltage (V)	Product ion (m/z)	Collision energy (V)	Ion mode
DRPM	421.15	26	274.04	18	ES+
DRPM-d_4	424.98	20	346.12	14	ES+
MEPM	384.16	24	140.91	18	ES+
MEPM-d_6	390.21	24	147.01	16	ES+
CPFX	332.14	28	231	36	ES+
CPFX-d_8	340.2	38	253.03	42	ES+
LVFX	362.14	30	261.1	26	ES+
LVFX-d_8	370.2	36	265.19	32	ES+
PZFX	319.1	22	284.05	18	ES+
PZFX-d_4	322.97	20	288.07	20	ES+
LZD	338.01	30	235.02	20	ES+
LZD-d_3	341.2	34	235.09	20	ES+
TZD	371	28	343.06	18	ES+
TZD-d_3	374.07	30	346.12	18	ES+

Abbreviations: CPFX, ciprofloxacin; DRPM, doripenem; LVFX, levofloxacin; LZD, linezolid; MEPM, meropenem; PZFX, pazufloxacin; TZD, tedizolid.

2.7.1 | Accuracy and precision

Accuracy and precision were calculated and expressed as relative error (RE%) and coefficient of variation (CV%), respectively, for each analytical batch (within-batch) and for three validation batches (batch-to-batch). Each validation batch contained eight calibrators and 6 replicates of QC samples at four levels (LLOQ, QC A, QC B, and QC C). The acceptance criterion for precision was below 15% CV for QCs (but 20% for LLOQ), and that for accuracy was between −15 and 15% for QCs (but between −20% and 20% for LLOQ).

2.7.2 | Recovery rate and matrix effect

Recovery rates were evaluated by comparing the peak areas of QC A, QC B, and QC C with those of deproteinized blank filtrate samples prepared from plasma samples of three different healthy volunteers spiked at the respective QC levels.

Matrix effects were evaluated by comparing the peak areas of deproteinized blank filtrate samples of three different healthy volunteers spiked at QC levels of A, B, and C with those of matrix-free solutions containing 100% standard and IS. Matrix effect was IS-compensated.

2.7.3 | Selectivity

Selectivity was analyzed using blank filtrate samples prepared from plasma samples of six different volunteers, without adding any standard solution or IS solution. The baseline signals at the expected retention time of each drug were evaluated as interfering peaks.

2.7.4 | Stability

Freeze–thaw stability was evaluated using QC A and QC B in triplicate measurement. Fifty microliters of blank filtrate was spiked with 5 μl of a QC sample (QC A or QC B) at 10×concentration. Three cycles of freezing at −40°C for 2 h and thawing at room temperature were performed. Then, 45 μl of 50% MeOH, 25 μl of IS, and 100 μl of acetonitrile were added. Stability was evaluated by calculating the precision and accuracy of the QC samples.

Autosampler stability was evaluated by calculating the precision and accuracy of QC A, QC B, and QC C samples analyzed after storage in an autosampler at 4°C for 24 or 72 h.

2.7.5 | Calculation and linearity

The calibration curve was constructed using analyte-specific MRM quantifier transitions. The analysis response was calculated as a ratio of the peak area of individual analyte to that of the corresponding IS. Using the TargetLynx V4.1 software package (Waters), weighted linear regression (1/x) for each concentration and analytical response was calculated for each analytical batch. Linearity was confirmed by calculating the concentrations of calibrators back-calculated from the calibration curves, and RE%.

2.8 | Calculation of adsorption on hemofilter

The adsorption (Ads) rate on hemofilter was calculated using the following equations, as reported previously[18]:

$$Ads = (Ci \times Qpli) - [(Co \times Qplo) - (Co \times Qplo) + Cuf \times Quf].$$

$$Ads\ (\%) = Ads\ (\mu g/min)/(Ci \times Qbi) \times 100.$$

$$Qpli = Qbi \times (1 - Ht/100).$$

$$Qplo = Qpli - Quf = Qbi \times (1 - Ht/100) - Quf.$$

where Ci and Co are the plasma concentrations on the filter inlet side and filter outlet side, respectively; Cuf is the filtrate concentration; Qbi is the blood flow rate on the filter inlet side; Quf is the ultrafiltration flow rate (ml/min) on the filter inlet side; and Qpli and Qplo are the plasma flow rates on the filter inlet side and filter outlet side, respectively. The plasma concentrations of DRPM, MEPM, LZD, TZD, CPFX, LVFX, and PZFX were measured by the UHPLC–MS/MS method described by Kai et al.,[26]

3 | RESULTS AND DISCUSSION

3.1 | Mass spectrometric and chromatographic characteristics

During MS tuning, $[M+H]^+$ signals in positive ion mode with the highest intensity were selected. The mass spectra of precursor ions and product ions of the seven drugs are shown in Supplemental Figure S1. The precursor ion was automatically selected by MRM analysis, and the product ion with the highest sensitivity and specificity was selected. Figure 1 shows the chromatograms of blank filtrate and LLOQ, and Supplemental Figure S2 shows the chromatograms of QC C and patient samples. The retention time was 1.86 min for DRPM, 1.93 min for MEPM, 1.98 min for CPFX, 1.97 min for LVFX, 1.98 min for PZFX, 2.18 min for LZD, and 2.23 min for TZD. The total measurement time per sample was 7 min. The peaks in LLOQ were single and sharp. Signal-to-noise ratio (S/N) was ≥165, which was higher than that recommended in the FDA guidance (S/N ≥ 10). No interference peak was observed at the retention times of all the drugs in all the blank filtrate samples prepared from six different volunteers, confirming good selectivity.

3.2 | Full validation

Supplemental Figure S3 shows the linearity of the seven drugs. The correlation coefficients (R^2) for the drugs calculated by linear regression were as follows: $R^2 \geq 0.9981$ for DRPM (calibration range: 0.2–100 μg/ml), $R^2 \geq 0.9934$ for MEPM (calibration range: 0.5–250 μg/ml), $R^2 \geq 0.9953$ for CPFX (calibration range: 0.1–50 μg/ml), $R^2 \geq 0.9967$ for LVFX (calibration range: 0.2–100 μg/ml), $R^2 \geq 0.9971$ for PZFX

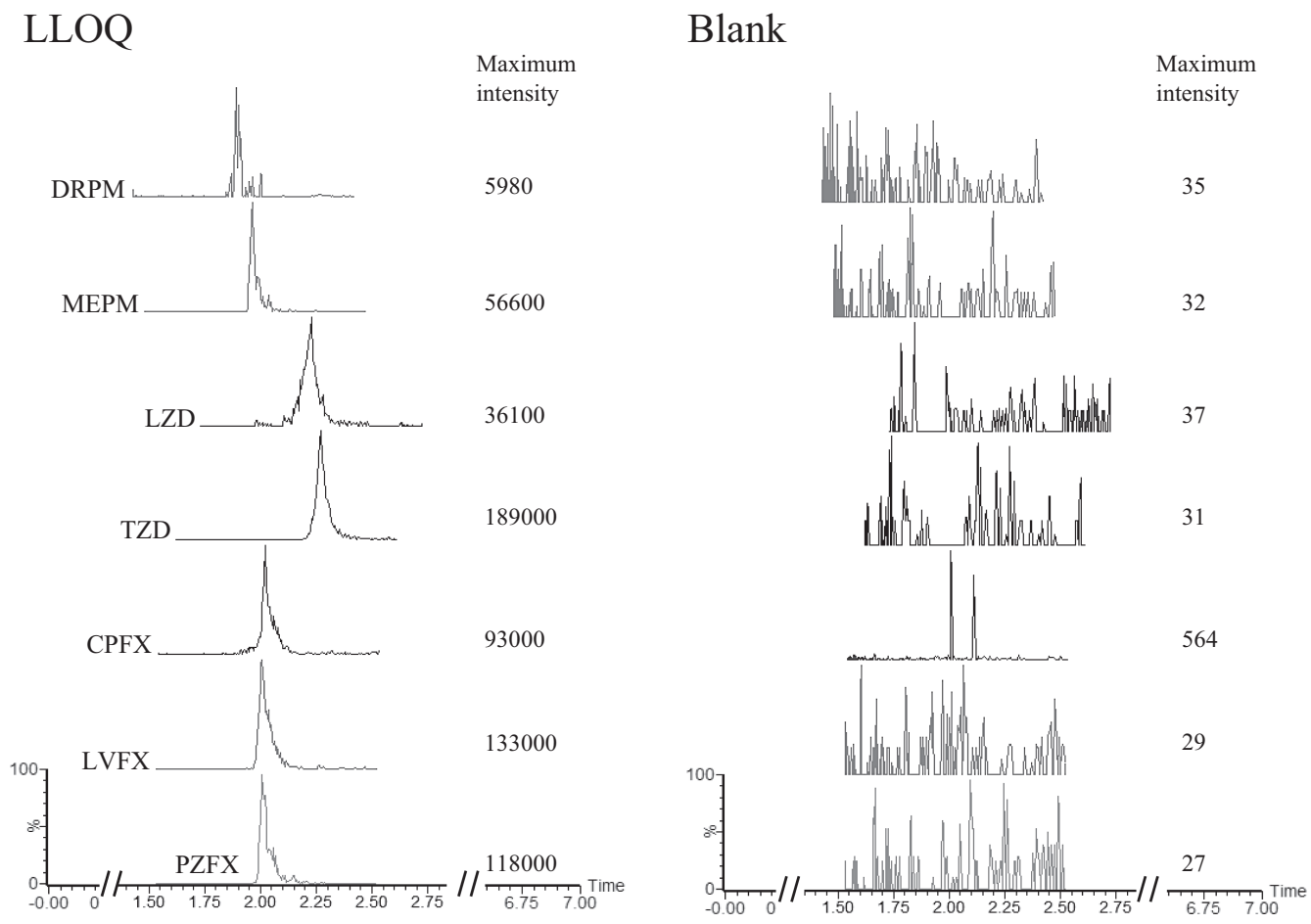

FIGURE 1 Chromatograms for measurement of doripenem (DRPM), meropenem (MEPM), linezolid (LZD), tedizolid (TZD), ciprofloxacin (CPFX), levofloxacin (LVFX), and pazufloxacin (PZFX) in lower limit of quantitation (LLOQ) filtrate sample (left) compared with blank filtrate (right).

TABLE 2 Validation results (accuracy and precision) for measuring filtrate concentrations of (A) DRPM, MEPM, CPFX, and LVFX. (B) PZFX, LZD, and TZD.

		Nominal DRPM concentrations (μg/ml)				Nominal MEPM concentrations (μg/ml)			
		LLOQ	QC A	QC B	QC C	LLOQ	QC A	QC B	QC C
(A)		0.2	0.6	6	75	0.5	1.5	15	187.5
Within-batch									
1	Mean (ng/ml)	0.19	0.57	6.41	72.73	0.49	1.53	16.65	177.25
	Precision (% CV)	10.98	7.07	6.90	3.59	9.91	4.70	1.85	6.57
	Accuracy (%)	−5.40	−5.71	6.89	−3.03	−2.10	1.70	11.03	−5.47
2	Mean (ng/ml)	0.21	0.57	6.20	71.01	0.51	1.49	16.44	194.50
	Precision (% CV)	10.23	8.55	5.27	4.90	5.65	6.06	2.25	3.13
	Accuracy (%)	3.60	−5.54	3.37	−5.32	1.05	−0.71	9.61	3.73
3	Mean (ng/ml)	0.19	0.58	6.21	75.94	0.50	1.42	14.87	181.55
	Precision (% CV)	11.06	5.94	4.83	3.95	4.06	3.02	3.26	6.03
	Accuracy (%)	−4.70	−2.52	3.50	1.26	−0.01	−5.44	−0.89	−3.17
Batch-to-batch	Mean (ng/ml)	0.20	0.57	6.28	73.34	0.50	1.47	15.87	185.33
	Precision (% CV)	10.65	6.87	5.47	4.67	6.09	5.37	5.87	6.30
	Accuracy (%)	−2.17	−4.51	4.59	−2.21	−0.21	−1.96	5.79	−1.16
		Nominal CPFX concentrations (μg/ml)				Nominal LVFX concentrations (μg/ml)			
		LLOQ	QC A	QC B	QC C	LLOQ	QC A	QC B	QC C
		0.1	0.3	3	37.5	0.2	0.6	6	75
Within-batch									
1	Mean (ng/ml)	0.10	0.28	2.90	36.25	0.20	0.55	5.57	74.48
	Precision (% CV)	4.76	2.89	3.45	3.60	6.62	3.49	1.39	4.25
	Accuracy (%)	1.32	−7.23	−3.23	−3.32	−0.35	−7.72	−7.16	−0.69
2	Mean (ng/ml)	0.10	0.29	3.03	37.37	0.20	0.57	5.54	72.80
	Precision (% CV)	7.15	4.12	6.37	8.41	4.85	1.42	2.95	4.90
	Accuracy (%)	−4.08	−4.57	1.13	−0.33	1.24	−5.72	−7.71	−2.93
3	Mean (ng/ml)	0.10	0.29	3.01	37.00	0.20	0.55	5.64	76.14
	Precision (% CV)	6.64	1.88	2.09	5.28	4.63	1.96	2.36	3.16
	Accuracy (%)	−0.25	−3.34	0.33	−1.34	−0.45	−8.81	−6.05	1.52
Batch-to-batch	Mean (ng/ml)	0.10	0.28	2.98	36.91	0.20	0.56	5.58	74.47
	Precision (% CV)	6.37	3.40	4.67	6.01	5.17	2.71	2.31	4.32
	Accuracy (%)	−1.14	−5.05	−0.64	−1.57	0.15	−7.33	−6.97	−0.70

		Nominal PZFX concentrations (μg/ml)				Nominal LZD concentrations (μg/ml)				Nominal TZD concentrations (μg/ml)			
		LLOQ	QC A	QC B	QC C	LLOQ	QC A	QC B	QC C	LLOQ	QC A	QC B	QC C
(B)		0.2	0.6	6	75	0.2	0.6	6	75	0.02	0.06	0.6	7.5
Within-batch													
1	Mean (ng/ml)	0.21	0.64	6.34	79.86	0.19	0.55	6.00	70.36	0.02	0.06	0.60	7.28
	Precision (% CV)	7.58	5.22	2.18	5.95	6.24	4.19	2.05	2.31	2.73	2.80	1.65	3.63
	Accuracy (%)	2.80	6.48	5.70	6.48	−6.10	−7.85	0.07	−6.19	−6.65	−6.24	−0.55	−2.93
2	Mean (ng/ml)	0.20	0.62	6.40	77.97	0.18	0.60	6.11	77.48	0.02	0.06	0.59	7.33
	Precision (% CV)	4.15	2.27	4.52	3.70	3.52	4.01	2.83	2.36	4.10	1.58	4.15	2.71
	Accuracy (%)	2.17	3.03	6.74	3.96	−9.01	0.12	1.87	3.30	−8.00	−5.83	−2.40	−2.30

TABLE 2 (Continued)

		Nominal PZFX concentrations (μg/ml)				Nominal LZD concentrations (μg/ml)				Nominal TZD concentrations (μg/ml)			
		LLOQ	QC A	QC B	QC C	LLOQ	QC A	QC B	QC C	LLOQ	QC A	QC B	QC C
(B)		0.2	0.6	6	75	0.2	0.6	6	75	0.02	0.06	0.6	7.5
3	Mean (ng/ml)	0.21	0.62	6.40	80.42	0.19	0.55	5.99	73.73	0.02	0.06	0.59	7.28
	Precision (% CV)	4.51	4.05	2.49	1.69	4.42	1.39	2.43	2.76	5.01	3.07	1.58	2.18
	Accuracy (%)	6.21	3.58	6.64	7.22	−5.49	−8.29	−0.10	−1.69	−4.50	−8.28	−1.18	−2.99
Batch-to-batch	Mean (ng/ml)	0.21	0.63	6.38	79.42	0.19	0.57	6.04	73.85	0.02	0.06	0.59	7.29
	Precision (% CV)	5.41	4.17	3.13	4.14	4.98	5.41	2.49	4.68	4.14	2.68	2.67	2.75
	Accuracy (%)	3.78	4.44	6.39	5.89	−6.67	−5.17	0.61	−1.53	−6.38	−6.78	−1.38	−2.74

Abbreviations: CPFX, ciprofloxacin; DRPM, doripenem; LVFX, levofloxacin; LZD, linezolid; MEPM, meropenem; PZFX, pazufloxacin; TZD, tedizolid.

(calibration range: 0.2–100 μg/ml), $R^2 \geq 0.9990$ for LZD (calibration range: 0.2–100 μg/ml), and $R^2 \geq 0.9994$ for TZD (calibration range: 0.02–10 μg/ml). The relative errors calculated from the calibration curves were <15% for all the drugs, indicating good linearity. Carryover was evaluated by injecting blank samples three times after measuring the highest concentration in the calibration range. All drugs fulfilled the validation criteria of FDA guidance for carryover (20% or less of the analyte and 5% or less of the internal standard at LLOQ) (Data not shown).

Table 2 shows the validation results for precision and accuracy of the seven drugs. For all the drugs, the results of precision and accuracy fulfilled the acceptance criteria of FDA guidance: for LLOQ, precision was ≤11.06% CV (within-batch) and ≤ 10.65% CV (batch-to-batch), and accuracy was ≤|9.01%| (within-batch) and ≤ |6.67%| (within-batch); for QCs, precision was ≤8.55% CV (within-batch) and ≤6.87% CV (within-batch), and accuracy was ≤|11.03%| (within-batch) and ≤ |7.33%| (within-batch).

Table 3 shows the recovery rate, matrix effect, as well as freeze–thaw and autosampler stability for the seven drugs. Recovery rate was ≥87.20% for DRPM, ≥94.68% for MEPM, ≥104.61% for CPFX, ≥96.18% for LVFX, ≥97.15% for PZFX, ≥95.58% for LZD and ≥96.93% for TZD. Matrix effect was 102.91%–115.60% for DRPM, 108.59%–114.50% for MEPM, 101.63%–105.93% for CPFX, 108.58%–114.46% for LVFX, 99.57–107.08% for PZFX, 100.00%–104.53% for LZD, and 103.83%–109.83% for TZD. Recovery rate and matrix effect were not remarkably different between QCs for all the drugs. Our assay system was designed to simultaneously measure several drugs with different properties. However, it was impossible to extract all the drugs efficiently at the same time using solid-phase extraction. Therefore, deproteinization was selected as the extraction method. In general, the deproteinization method is selected in simultaneous measurement systems because the extraction step is simple.[19,22,23,25] However, extraction by the deproteinization method may result in interference of ionization such as ion suppression and ion enhancement.[28] In addition, since the cone is more easily contaminated when using deproteinization compared with solid-phase extraction, frequent maintenance is required. On the contrary, since the CRRT filtrate contains only the components after being filtered by the hemofilter, it is considered that deproteinized filtrate samples may not contain a large quantity of biological substances, unlike plasma that contains a large amount of matrix. In fact, ion suppression and ion enhancement were slight, resulting in good linearity and sharp peaks even at LLOQ. When QCA and QC B were subjected to three freeze–thaw cycles, precision was ≤6.60% CV and accuracy was ≤|8.70%|. After placing the three QC samples in autosampler at 4°C for 24 h, precision was ≤6.14% CV and accuracy was ≤|12.67%|, and those for 72 h were ≤5.60% and ≤|14.56%|, respectively.

3.3 | Comparison with reported methods

Because this is the first report of simultaneous quantification of multiple antibiotics in filtrate of CRRT circuit, comparison with other assay methods for filtrate is not possible. For quantification of plasma concentrations, we used a similar UHPLC–MS/MS assay that we previously reported for measuring multiple antimicrobials in plasma.[26] For this assay, the calibration ranges were 0.2–100 μg/ml for DRPM, 0.1–50 μg/ml for MEPM, 0.02–10 μg/ml for CPFX, 0.04–20 μg/ml for LVFX, 0.04–20 μg/ml for PZFX, 0.1–50 μg/ml for LZD, and 0.01–5 μg/ml for TZD.[26] Meanwhile, the ranges of calibration curves for measuring plasma concentrations using other previously reported methods were 0.05–100 μg/ml for DRPM,[20] 0.1–100 μg/ml for MEPM,[23] 0.025–30 μg/ml for CPFX,[19] 0.09–4.53 μg/ml for LVFX,[25] 0.02–0.5 μg/ml for PZFX,[22] 1–100 μg/ml for LZD,[24] and 0.00074–1.5 μg/ml for TZD.[21] Comparing our results with other reports, the concentration ranges of our calibration curves are relatively broad. Our pretreatment method is more straightforward and can extract multiple drugs in the same batch, whereas more complicated methods such as solid-phase extraction and ethanol precipitation extraction were used in other studies.[19–25] The run time of our method is also shorter (7 min[26]) than previous reports (8–22 min).[19–25] Our UHPLC–MS/MS assay yields sharper peaks[26] compared with some methods that used

TABLE 3 Validation results (stability, recovery rate, and matrix effect) for measuring filtrate concentrations of (A) DRPM, MEPM, CPFX, and LVFX. (B) PZFX, LZD, and TZD.

	Nominal DRPM concentrations (μg/ml)			Nominal MEPM concentrations (μg/ml)		
	QC A	QC B	QC C	QC A	QC B	QC C
(A)	0.6	6	75	1.5	15	187.5
Freeze–thaw stability						
Mean (ng/ml)	0.61	6.35		1.60	16.30	
Precision (% CV)	1.89	4.56		5.78	0.63	
Accuracy (%)	2.35	5.86		6.37	8.70	
Autosampler stability (24 h)						
Mean (ng/ml)	0.61	6.18	71.26	1.49	16.68	211.25
Precision (% CV)	3.85	2.10	4.23	6.14	1.13	3.05
Accuracy (%)	1.25	2.98	−4.98	−0.98	11.18	12.67
Autosampler stability (72 h)						
Mean (ng/ml)	0.60	6.87	71.04	1.51	16.71	194.39
Precision (% CV)	4.27	2.39	1.56	5.65	1.11	5.02
Accuracy (%)	−0.27	14.56	−5.28	0.49	11.39	3.67
Recovery rate (%)	87.20	97.96	105.20	94.68	99.06	97.49
Recovery rate (% CV)	10.01	1.77	5.15	0.39	4.98	2.25
Matrix effect (%)	102.91	115.60	103.33	114.50	108.59	115.71
Matrix effect (%CV)	11.40	2.06	2.20	7.74	5.52	1.17

	Nominal CPFX concentrations (μg/ml)			Nominal LVFX concentrations (μg/ml)		
	QC A	QC B	QC C	QC A	QC B	QC C
	0.3	3	37.5	0.6	6	75
Freeze-thaw stability						
Mean (ng/ml)	0.30	3.13		0.57	6.20	
Precision (% CV)	1.13	3.54		2.09	6.60	
Accuracy (%)	−1.60	4.30		−5.52	3.26	
Autosampler stability (24 h)						
Mean (ng/ml)	0.32	3.05	38.24	0.60	6.10	74.92
Precision (% CV)	2.74	4.53	3.75	4.25	4.23	2.72
Accuracy (%)	6.50	1.80	1.98	−0.30	1.71	−0.11
Autosampler stability (72 h)						
Mean (ng/ml)	0.29	3.09	38.18	0.57	5.57	71.23
Precision (% CV)	1.86	2.74	2.43	3.18	2.35	2.73
Accuracy (%)	−1.77	2.96	1.80	−4.45	−7.11	−5.02
Recovery rate (%)	106.51	104.61	109.17	100.96	96.18	92.72
Recovery rate (% CV)	1.64	2.97	6.74	2.75	2.42	5.10
Matrix effect (%)	105.93	101.63	102.29	114.46	108.58	112.04
Matrix effect (% CV)	8.43	0.83	4.12	3.98	1.70	2.41

	Nominal PZFX concentrations (μg/ml)			Nominal LZD concentrations (μg/ml)			Nominal TZD concentrations (μg/ml)		
	QC A	QC B	QC C	QC A	QC B	QC C	QC A	QC B	QC C
(B)	0.6	6	75	0.6	6	75	0.06	0.6	7.5
Freeze-thaw stability									
Mean (ng/ml)	0.63	6.38		0.62	6.41		0.06	0.61	

TABLE 3 (Continued)

	Nominal PZFX concentrations (μg/ml)			Nominal LZD concentrations (μg/ml)			Nominal TZD concentrations (μg/ml)		
	QC A	QC B	QC C	QC A	QC B	QC C	QC A	QC B	QC C
(B)	0.6	6	75	0.6	6	75	0.06	0.6	7.5
Precision (%CV)	1.15	2.06		1.53	0.40		2.96	1.56	
Accuracy (%)	4.44	6.41		3.77	6.78		−0.83	1.45	
Autosampler stability (24 h)									
Mean (ng/ml)	0.63	5.98	73.64	0.59	6.09	78.08	0.06	0.60	7.67
Precision (%CV)	3.95	3.24	2.76	2.94	4.62	3.05	0.69	1.58	1.61
Accuracy (%)	4.34	−0.30	−1.81	−1.89	1.52	4.10	−0.98	0.19	2.30
Autosampler stability (72 h)									
Mean (ng/ml)	0.63	6.26	77.38	0.59	5.81	72.83	0.06	0.60	7.59
Precision (%CV)	5.60	4.50	1.25	5.11	4.65	4.00	4.85	0.83	2.36
Accuracy (%)	4.50	4.42	3.18	−2.03	−3.17	−2.89	2.54	0.47	1.24
Recovery rate (%)	97.15	97.67	101.54	100.83	95.58	100.26	97.67	96.93	99.17
Recovery rate (%CV)	6.41	1.83	1.84	1.43	1.01	2.83	0.69	2.74	0.62
Matrix effect (%)	104.64	107.08	99.75	100.00	104.53	102.81	109.83	103.83	107.04
Matrix effect (%CV)	1.10	0.88	1.05	13.23	1.71	0.75	1.35	1.18	0.65

Abbreviations: CPFX, ciprofloxacin; DRPM, doripenem; LVFX, levofloxacin; LZD, linezolid; MEPM, meropenem; PZFX, pazufloxacin; TZD, tedizolid.

high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection.[20,22,23]

3.4 | Clinical application

To evaluate the clinical applicability of the developed method, drug concentrations in CRRT filtrates sampled 1 h after intravenous administration of antibiotics at more than 3 days from treatment initiation were measured. Table 4 shows the patient profile, filtrate concentrations, plasma concentrations of antibiotics, and adsorption rates of antibiotics on hemofilters. The concentrations in the filtrate were within the calibration curve ranges for all the drugs: 12.32–38.28 μg/ml (calibration range: 0.2–100 μg/ml) for DRPM, 19.38–53.99 μg/ml (calibration range: 0.5–250 μg/ml) for MEPM, 6.81–13.54 μg/ml (calibration range: 0.1–50 μg/ml) for CPFX, 7.50–10.52 μg/ml (calibration range: 0.2–100 μg/ml) for LVFX, 29.90–57.64 μg/ml (calibration range: 0.2–100 μg/ml) for PZFX, 12.29–25.83 μg/ml (calibration range: 0.2–100 μg/ml) for LZD, and 0.35–0.87 μg/ml (calibration range: 0.02–10 μg/ml) for TZD. Although it was difficult to identify the drugs adsorbed on the membranes because of the small samples (Table 4), the adsorption rates differed among the membranes, suggesting the need to adjust the dosage depending on the type of membrane. Negative adsorption rates were observed in some membranes. Previous reports have shown that levofloxacin is released after adsorption on PAN membranes,[29] suggesting that a similar phenomenon may have occurred.

As mentioned in Introduction, several antimicrobial agents have been known to adsorb on hemofilter, but no method that allows accurate determination of the amount of adsorption was available.[7–15] We report for the first time a UHPLC–MS/MS assay for simultaneous measurement of CRRT filtrate levels of seven antibiotics frequently used in ICU. In a previous study, we have established and validated a simultaneous quantification method for plasma concentrations of 12 antimicrobial agents,[26] which included the seven drugs investigated in the present study. Since the method for measuring CRRT filtrate concentrations and that for assaying plasma concentrations use the same extraction method and MS conditions, it would be possible to simultaneously measure drug concentrations in the filtrate and in the plasma easily. The measured blood and filtrate drug concentrations can then be used to calculate the amount of drug adsorption on hemofilters, which will provide important information on whether dose adjustment is necessary.

4 | CONCLUDING REMARKS

We report for the first time a UHPLC–MS/MS method for simultaneous quantification of CCRT filtrate concentrations of carbapenems (DRPM and MEPM), quinolones (CPFX, LVFX, and PZFX), and anti-MRSA agents (LZD and TZD) that are frequently used in ICU patients. This assay system is simple, rapid, and fulfills the criteria of FDA guidance. Antibiotic concentration in CRRT filtrate together with blood concentrations can be used to calculate the amount of

TABLE 4 Patient characteristics, filtrate concentrations, plasma concentrations, and hemofilter adsorption rates of DRPM, MEPM, CPFX, LVFX, PZFX, LZD, and TZD.

Substance	Gender	Age (years)	Weight (kg)	Height (cm)	Dosage (mg)	Dosing period (days)	Membrane	Filtrate concentration (μg/ml)	Plasma concentration on filter inlet side (μg/ml)	Plasma concentration on filter outlet side (μg/ml)	Adsorption (%)
DRPM	Female	52	43.2	151.7	500 (every 8 h)	3	AN69ST	38.28	25.68	30.12	−8.46
	Male	51	89.0	182.0	500 (every 8 h)	3	ATA	12.32	19.59	26.74	−31.60
	Male	71	60.1	171.0	1000 (every 8 h)	3	AN69ST	27.91	18.08	27.37	11.85
MEPM	Female	51	51.7	160.6	500 (every 6 h)	3	AN69ST	53.99	59.22	55.86	11.92
	Female	74	61.0	152.1	500 (every 6 h)	3	ATA	34.81	42.57	36.82	19.25
	Female	56	60.8	144.0	500 (every 6 h)	9	PES	19.38	28.27	30.29	−68.75
CPFX	Female	74	61.0	152.1	800 (once-daily)	3	PMMA	13.54	18.19	18.48	−7.72
	Male	71	87.4	168.0	800 (once-daily)	3	ATA	6.94	9.08	8.12	6.17
	Male	81	51.0	144.5	800 (once-daily)	3	AN69ST	6.81	10.88	13.03	11.21
LVFX	Male	51	89.0	182.0	500 (once-daily)	3	ATA	7.50	8.82	8.70	8.25
	Male	71	60.1	171.0	500 (once-daily)	3	ATA	10.32	5.04	6.59	−35.65
	Female	65	51.7	160.6	500 (once-daily)	3	PES	10.52	9.69	10.18	0.30
PZFX	Male	90	38.0	149.6	1000 (once-daily)	3	PMMA	44.88	48.73	49.65	3.10
	Male	78	61.8	159.8	1000 (once-daily)	3	ATA	29.90	35.54	31.67	9.35
	Female	65	51.7	160.6	1000 (once-daily)	3	AN69ST	57.64	64.25	73.20	−3.64
LZD	Male	71	60.1	171.0	600 (every 12 h)	3	AN69ST	14.06	7.49	10.61	−42.89
	Female	74	61.0	152.1	600 (every 12 h)	3	PMMA	25.83	29.04	30.16	2.29
	Male	72	87.4	168.0	600 (every 12 h)	3	ATA	12.29	9.08	11.76	−24.70
TZD	Female	65	51.7	160.6	200 (once-daily)	3	PMMA	0.87	5.52	5.88	6.38
	Male	71	60.1	171.0	200 (once-daily)	3	ATA	0.35	1.96	1.30	46.17
	Male	72	60.4	164.0	200 (once-daily)	3	AN69ST	0.39	2.77	2.77	14.15

Abbreviations: ATA, asymmetric cellulose triacetate; CPFX, ciprofloxacin; DRPM, doripenem; LVFX, levofloxacin; LZD, linezolid; MEPM, meropenem; PES, polyethersulfone; PMMA, polymethyl methacrylate; PZFX, pazufloxacin; TZD, tedizolid.

drug adsorption on hemofilters, providing important information on whether dose adjustment is required.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Raw data were generated at Oita University Hospital. Derived data supporting the findings of this study are available from the corresponding author Kai M on request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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