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Quantification of doripenem in human plasma and peritoneal fluid by high-performance liquid chromatography with ultraviolet detection

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ABSTRACT

A simple and rapid HPLC method that includes ultrafiltration to remove plasma and peritoneal fluid protein was developed to determine doripenem concentrations in human plasma and peritoneal fluid. Doripenem was stabilized by immediate mixing of the plasma or peritoneal fluid with 1 M 3-morpholinopropanesulfonic acid buffer (pH 7.0) (1:1). Doripenem and an internal standard were detected by measuring their ultraviolet absorbance at 300 nm. The calibration curves for doripenem in human plasma and peritoneal fluid were linear from 0.05 to 100 μg/mL. For plasma, both the intra- and the inter-day precision were less than 3.41% (CV), and the accuracy was between 97.4 and 101.7% above 0.05 μg/mL. For peritoneal fluid, the intra- and the interday precision were less than 2.98% (CV), and the accuracy was between 94.4 and 103.9% above 0.05 μg/mL. The limit of detection was 0.02 μg/mL in both plasma and peritoneal fluid. The assay has been applied to the therapeutic drug monitoring of doripenem in both plasma and peritoneal fluid.

1. Introduction

Doripenem (Fig. 1) is a novel carbapenem with antibacterial activity against a broad range of Gram-positive and Gram-negative bacteria. For example, against members of the family *Enterobacteriaceae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*, the MIC_{90} (MIC: minimum inhibitory concentration) range from 0.032 to 0.5 μg/mL [1]. Doripenem appears to be a potent carbapenem with a spectrum resembling currently marketed antipseudomonal carbapenems, but with greater activity when tested against some non-fermentative bacillary strains [2]. Furthermore, doripenem is stable against human renal dehydropeptidase-I [3], and thus, unlike imipenem, its use does not require co-administration of a dehydropeptidase-I enzyme inhibitor such as cilastatin.

Doripenem has been used clinically for the treatment of intra-abdominal infections as well as for antibacterial prophylaxis in abdominal surgery [4,5]. Although the concentration of a drug in its target tissue is a key determinant of its efficacy, especially for antibiotics, therapeutic drug monitoring of doripenem has mainly been carried out using plasma. As one reason for this is the lack of a reliable determination method, it is important to develop a determination method for doripenem not only for plasma but also for peritoneal fluid to monitor concentrations of this drug.

A microbiological assay has been widely used for measuring the plasma levels of doripenem. However, microbiological assays cannot differentiate doripenem from other antibiotics that may have been co-administered, and an HPLC method would be preferable due to its specificity. Recently, Sutherland and Nicolau reported an HPLC method involving solid extraction of human and mouse serum [6]. However, the method does not have a sufficiently low limit of quantification (0.5 μg/mL) and requires long sample preparation times.

Other carbapenems, such as imipenem, biapenem, and meropenem, have been measured in plasma using HPLC and ultrafiltration [7–9]. Applying this ultrafiltration method to deproteinization, we have developed a simple and rapid HPLC method for measuring doripenem in both plasma and peritoneal fluid.

2. Experimental

2.1. Reagents and materials

Standard doripenem was provided by Shionogi & Co., Ltd. (Osaka, Japan). Meropenem as the internal standard (IS) was provided by Dainippon Sumitomo Pharma Co., Ltd. (Osaka, Japan). 1 M 3-morpholinopropanesulfonic acid (MOPS) buffer (pH 7.0), often used as a stabilizer for carbapenems [7–9], was purchased

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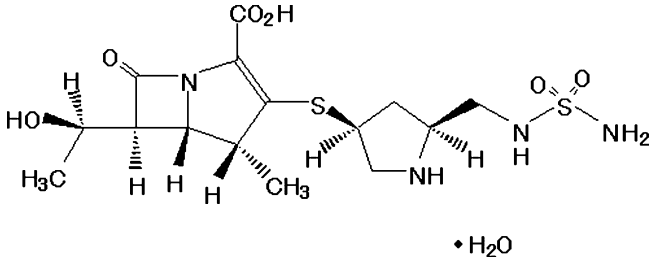

Fig. 1. Structure of doripenem.

from Dojindo Laboratories (Kumamoto, Japan). $Na_2HPO_4{\cdot}12H_2O$ and $NaH_2PO_4{\cdot}2H_2O$ were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and acetonitrile was purchased from Sigma–Aldrich Japan (Tokyo, Japan). All other chemicals were of analytical grade. The Nanosep 10 K centrifugal filter device was purchased from the Pall Corporation (New York, USA).

2.2. Equipment

The HPLC system consisted of a 600E system controller, a 700 Satellite WISP auto-sampler (Waters Corporation, Milford, MA, USA), an SPD-6A UV spectrophotometric detector (Shimadzu Corporation, Kyoto, Japan), a Chromatocorder 21 (System Instruments Co., Ltd., Tokyo, Japan), and a U-620 Type30 column heater (Sugai Chemical Industry Co., Ltd., Wakayama, Japan). The levels of albumin and total protein in plasma and peritoneal fluid were assayed using an S40 Clinical Analyzer (Hitachi Ltd., Tokyo, Japan).

2.3. Chromatographic conditions

The samples were separated by chromatography on an XBridge C18 5 μm (4.6 mm × 150 mm) column (Waters Corporation, Milford, MA, USA). The mobile phase was a mixture of 50 mM sodium phosphate buffer (pH 3.2) and acetonitrile (935:65, v/v), and the pump flow rate was 1.0 mL/min. The auto sampler was set to 4 °C, and the injection volume was 20 μL. The column temperature was 40 °C. The doripenem and IS peaks were detected by ultraviolet absorbance at 300 nm.

2.4. Plasma samples

Blood samples were collected into heparinized vacuum tubes (Nipro Corporation, Osaka, Japan), and separated by centrifugation at 1000 × *g* for 10 min at 4 °C. Plasma samples were mixed with the same volume of 1 M MOPS buffer and stored at −40 °C until analyzed. Control human plasma was a mixture of equal volumes of plasma from six healthy volunteers and stored at −40 °C.

2.5. Peritoneal fluid samples

Peritoneal fluid samples were mixed with the same volume of 1 M MOPS buffer and stored at −40 °C until analysis. Control peritoneal fluid was a mixture of equal volumes of peritoneal fluid from six patients who were not given doripenem, and the fluid was stored at −40 °C.

2.6. Analytical procedure

A working stock solution of doripenem was prepared daily at a concentration of 1 mg/mL in 50 mM MOPS buffer. Control plasma was spiked with doripenem, with the final concentrations corresponding to 0.05, 0.1, 0.5, 1.0, 5.0, 25.0, 50.0, 75.0, and 100.0 μg/mL. Samples (200 μL) were then mixed with 200 μL of 1 M MOPS buffer and 20 μL of 40 μg/mL IS solution and transferred to a Nanosep 10 K centrifugal filter device. Clinical plasma samples, which were mixed with the same volume of 1 M MOPS buffer and stored at −40 °C, were thawed to room temperature. A 400 μL aliquot was mixed with 20 μL of a 40 μg/mL IS solution and transferred to a Nanosep 10 K device. The devices were centrifuged at 12,000 × *g* for 10 min at room temperature. Filtrate (20 μL) was injected into the HPLC system for analysis. The peritoneal fluid samples were processed as well as the plasma.

2.7. Method validation

A calibration curve was made using the ratio of the observed peak height of doripenem and IS. Linear regression analysis of the calibration data was performed using the equation $y = mx + b$, with a weighting of $1/y$ where y is the peak area ratio, x is the concentration of doripenem, and m and b are the slope and intercept, respectively.

The method was evaluated for linearity, specificity, accuracy, and precision (expressed as the percent coefficient of variation [CV (%)]). Plasma standard samples (0.05, 0.1, 0.5, 1.0, 5.0, 25.0, 50.0, 75.0, and 100.0 μg/mL) were prepared using control plasma or control peritoneal fluid, and intra- and interday assay precision and accuracy were determined. The limit of detection (LOD) of doripenem was determined from the peak and standard deviation of the noise level, S_N. The LOD was defined as the sample concentration of doripenem that resulted in peak heights threefold higher than the S_N. The limit of quantification (LOQ) of doripenem was determined from validation data.

2.8. Recovery

The recovery of doripenem from plasma by ultrafiltration was determined by comparing the peak heights from plasma standards with those from doripenem standards that were similarly prepared except that an aqueous doripenem solution replaced the spiked control plasma (frozen–thawed sample) and the standard solutions were not filtered. The recovery from peritoneal fluid was determined, as well as that from plasma.

2.9. Specificity

To evaluate the method's specificity, six blank plasma samples from healthy volunteers and six blank peritoneal fluid samples from patients who were not given doripenem were investigated for interference by endogenous matrix components. Specificity was also assessed in the presence of other ß-lactams: biapenem, imipenem, cefepime, cefozopran, cefotiam, flomoxef, or cefmetazole at a concentration of 20 μg/mL.

2.10. Stability

The stability of the plasma samples and peritoneal fluid samples at 1.0, 5.0, and 50.0 μg/mL of doripenem was examined using an equal volume of 1 M MOPS buffer as a stabilizer after storage at −40 °C. Control plasma and control peritoneal fluid samples were spiked to contain 1.0, 5.0, and 50.0 μg/mL of doripenem. An equal volume of 1 M MOPS buffer was added, and the samples were mixed, separated into 0.5 mL aliquots, and stored at −40 °C. The doripenem concentration was determined at 0, 7, 15, and 30 days ($n = 3$).

The freeze–thaw stabilities of plasma and peritoneal fluid samples containing 1.0, 5.0, and 50.0 μg/mL of doripenem were also examined using an equal volume of 1 M MOPS buffer. The samples were stored at −40 °C for 24 h, completely thawed at room temperature (25 °C), and then refrozen at −40 °C for 24 h. The freeze–thaw

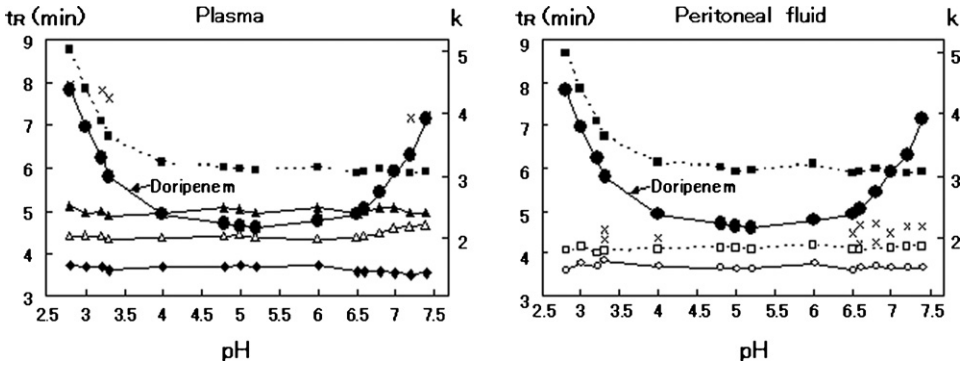

Fig. 2. The effect of the mobile phase pH on the retention time (t_R) in minutes and the retention factors (k) of the doripenem peak and endogenous peaks of plasma or peritoneal fluid. The retention times and retention factors were determined using 50 mM sodium phosphate solution–acetonitrile (95:5, v/v). Closed circles and solid line: doripenem; other closed symbols: peaks of endogenous compounds; open symbols and crosses: minute peaks of endogenous compounds.

cycle was repeated two more times, and the samples were analyzed after the third cycle ($n = 3$).

2.11. Application to pharmacokinetic studies in patients

Abdominal-surgery patients received a 0.5 h infusion of doripenem (500 mg) every 8 h. Plasma and peritoneal fluid concentrations of doripenem were measured at 0, 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, and 6.5 h after starting the infusion. Changes in the plasma and peritoneal fluid doripenem concentrations were fitted to a three-compartment model and analyzed with the nonlinear least-squares computer program (MULTI-Win) [10]. The exposure time during which the drug concentration remained at the MIC for microorganisms, $T > \text{MIC}$ (% of 24 h), for these patients was determined [11], because the efficiency of doripenem is related to the $T > \text{MIC}$.

Table 1
Accuracy and intra- and interday precision data for the measurement of doripenem in human plasma and peritoneal fluid

Concentration added (μg/mL)	Concentration found (mean ± S.D.) (μg/mL)	CV (%)	Accuracy (%)
Plasma			
Intraday assay ($n = 6$)			
0.05	0.050 ± 0.002	3.41	99.6
0.1	0.098 ± 0.002	2.26	97.5
0.5	0.503 ± 0.003	0.57	100.5
1.0	1.016 ± 0.005	0.45	101.6
5.0	5.084 ± 0.036	0.71	101.7
25.0	25.14 ± 0.181	0.72	100.6
50.0	49.70 ± 0.498	1.00	99.4
100.0	100.47 ± 0.764	0.76	100.5
Interday assay ($n = 6$)			
0.05	0.050 ± 0.001	2.71	99.7
0.1	0.097 ± 0.002	2.25	97.4
0.5	0.504 ± 0.007	1.39	100.7
1.0	1.011 ± 0.006	0.57	101.1
5.0	5.074 ± 0.062	1.23	101.5
25.0	25.08 ± 0.210	0.84	100.3
50.0	49.86 ± 0.116	0.23	99.7
100.0	100.16 ± 0.232	0.23	100.2
Peritoneal fluid			
Intraday assay ($n = 6$)			
0.05	0.047 ± 0.001	2.98	94.4
0.1	0.978 ± 0.003	2.66	97.8
0.5	0.514 ± 0.006	1.16	102.8
1.0	1.009 ± 0.008	0.75	100.9
5.0	5.195 ± 0.048	0.91	103.9
25.0	25.01 ± 0.434	1.73	100.1
50.0	50.96 ± 0.802	1.57	101.9
100.0	98.99 ± 0.767	0.78	99.0
Interday assay ($n = 6$)			
0.05	0.049 ± 0.001	2.63	98.7
0.1	0.099 ± 0.002	1.85	99.2
0.5	0.499 ± 0.008	1.56	99.9
1.0	1.008 ± 0.010	1.01	100.8
5.0	5.067 ± 0.082	1.63	101.3
25.0	24.99 ± 0.384	1.54	100.0
50.0	50.26 ± 0.919	1.83	100.5
100.0	100.09 ± 0.725	0.73	100.1

S.D.: standard deviation. CV (%): coefficient of variation.

Table 2
Analytical and statistical parameters for measurement of doripenem in human plasma and peritoneal fluid ($n = 6$)

	Calibration curve ($y = mx + b$)[a]			Range (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)
	Slope m (mean ± S.D.)	Intercept b (mean ± S.D.)	Correlation coefficient r^2 (mean ± S.D.)			
Plasma	55081 ± 1737	1299 ± 845	$0.9999 \pm 7.0 \times 10^{-5}$	0.05–100	0.02	0.05
Peritoneal fluid	54828 ± 1569	1392 ± 1029	$0.9999 \pm 7.0 \times 10^{-5}$	0.05–100	0.02	0.05

[a] $y = mx + b$ with a weighting of $1/y$; y the peak area ratio; x, the concentration of doripenem; m the slope; b the intercept.

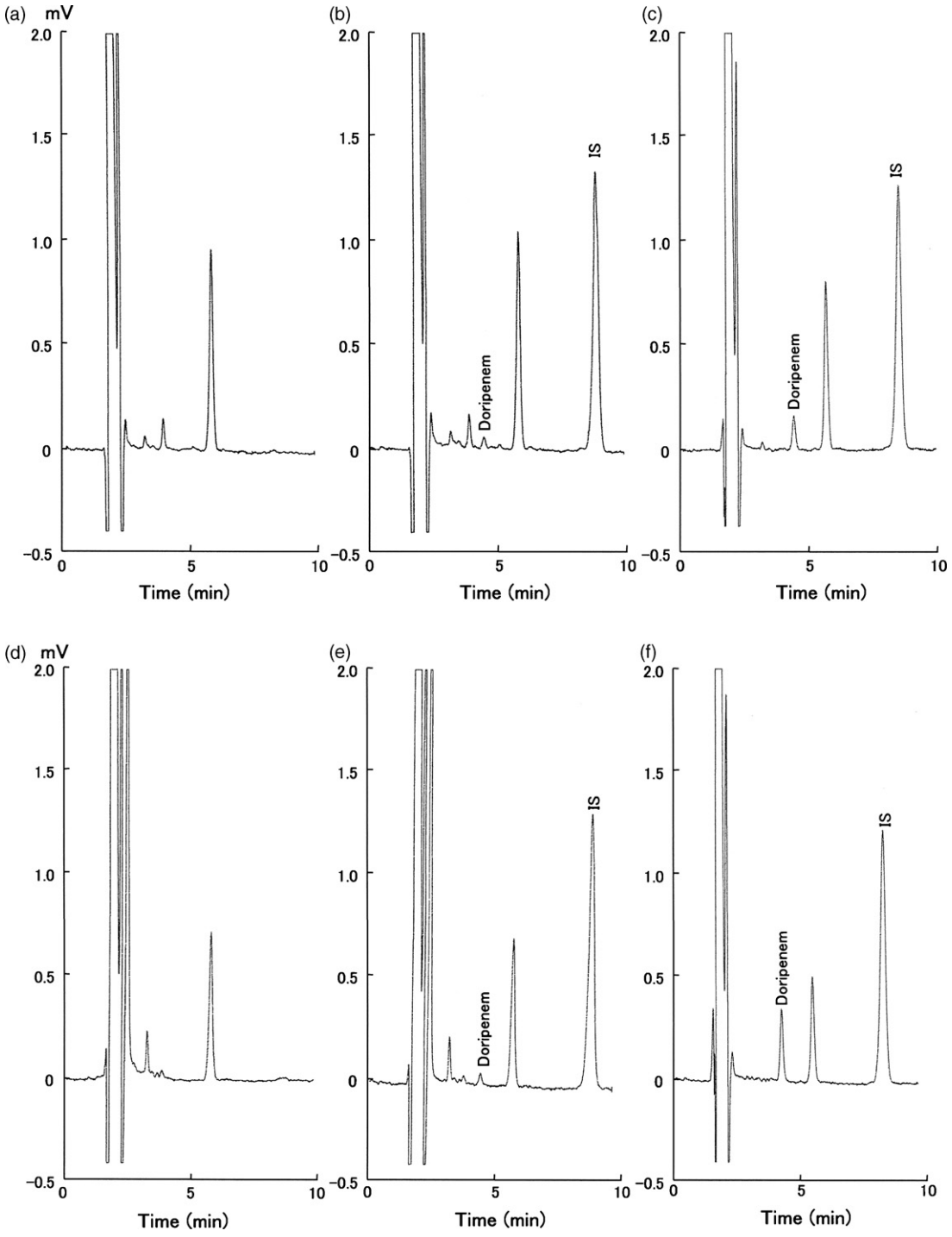

Fig. 3. Typical chromatograms: (a) blank control plasma; (b) control plasma spiked with 0.10 μg/mL doripenem; (c) patient plasma sample (0.30 μg/mL); (d) blank control peritoneal fluid; (e) control peritoneal fluid spiked with 0.10 μg/mL doripenem; (f) Patient peritoneal fluid sample (0.68 μg/mL).

3. Results

3.1. Chromatographic conditions

Preliminary studies using sodium phosphate buffer, sodium acetate buffer, acetonitrile, and methanol suggested that the pH of the mobile phase was important for the HPLC and that considerable buffer concentrations were needed to maintain the optimal pH. Thus, the optimal pH of the mobile phase for separation of the doripenem peak from endogenous peaks in the plasma or peritoneal fluid profiles was determined using 50 mM sodium phosphate solution–acetonitrile (95:5, v/v). Fig. 2 indicates the retention times (t_R) in minutes and the retention factors (k) of doripenem and endogenous compounds in plasma or peritoneal fluid. A pH of 3.2 was selected for the mobile phase, and the acetonitrile content was increased for appropriate analysis times. Finally, the optimal mobile phase was determined to be a mixture of 50 mM sodium phosphate buffer (pH 3.2) and acetonitrile (935:65, v/v).

3.2. Typical chromatograms

Fig. 3 illustrates a biological matrix with blank control plasma (Fig. 3a) and control plasma spiked with 0.10 μg/mL of doripenem (Fig. 3b), as well as a 6.5 h plasma sample from patient A with a concentration of 0.30 μg/mL (Fig. 3c). A biological matrix with blank control peritoneal fluid (Fig. 3d) and blank control peritoneal fluid spiked with 0.10 μg/mL of doripenem (Fig. 3e) are also illustrated, as well as a 6.5 h peritoneal fluid sample from patient A with a concentration of 0.68 μg/mL (Fig. 3f). Interfering peaks were not evident and the retention time for doripenem was 4.3 min.

3.3. Method validation

Table 1 summarizes the reproducibility and accuracy at each calibration standard from 0.05 to 100 μg/mL. For both the intra- and interday assay of doripenem in plasma and peritoneal fluid, all CV values were ≤3.41% and accuracy values were 94.4–103.9%.

3.4. Limits of detection and quantification

The LOD of both plasma and peritoneal fluid, defined as the concentration of doripenem giving a signal-to-noise ratio of >3:1, was 0.02 μg/mL, using a 20 μL injection volume. Because all the precision and accuracy data were <10% (Table 1), the LOQ was determined as the lowest concentration of standard used (0.05 μg/mL [plasma: CV: 3.41%, accuracy: 99.6%, intraday assay, n = 6; peritoneal fluid: CV: 2.98%, accuracy: 94.4%, intraday assay, n = 6]). Table 2 summarizes the analytical and statistical parameters for the measurement of doripenem in human plasma and peritoneal fluid.

3.5. Recovery

For plasma, the recoveries of doripenem (n = 6) at 1.0, 5.0, 25.0, and 100.0 μg/mL were 91.8 ± 0.9%, 91.2 ± 0.6%, 92.8 ± 0.7%, and 91.3 ± 0.6%, respectively. For peritoneal fluid, the recoveries (n = 6) at the same concentrations were 88.5 ± 1.0, 90.9 ± 1.4, 90.7 ± 0.5, and 90.0 ± 0.5%, respectively.

Table 3 shows the recovery of doripenem from various plasma and peritoneal fluid samples. The recovery tended to increase at lower concentrations of plasma and peritoneal fluid (100% < 50% dilution <25% dilution). The recovery values were similar among subjects A–E (plasma) and subjects F–J (peritoneal fluid).

3.6. Specificity

Six blank plasma samples and six peritoneal fluid samples were investigated for interference by endogenous matrix components, and no interference peak was observed. Specificity was assessed in the presence of other ß-lactams: biapenem, imipenem, cefepime, cefozopran, cefotiam, flomoxef, or cefmetazole at a concentration of 20 μg/mL. Neither chromatogram revealed any limitations for the assay.

3.7. Stability

The stability of the plasma samples at 1.0, 5.0, and 50.0 μg/mL of doripenem was examined using an equal volume of 1 M MOPS buffer as a stabilizer after storage at −40 °C. The mean concentrations (±S.D.; n = 3 for each) at 30 days were 104.2 ± 0.7% (n = 3), 99.1 ± 0.6%, and 99.0 ± 0.4% of the initial concentration at 1.0, 5.0, and 50.0 μg/mL, respectively. The stability of the peritoneal fluid samples at 1.0, 5.0, and 50.0 μg/mL of doripenem was also examined, using an equal volume of 1 M MOPS buffer as a stabilizer after storage at −40 °C. The mean concentrations (±S.D.; n = 3 for each) at 30 days were 97.9 ± 0.4%, 98.3 ± 0.7%, and 100.0 ± 0.2% of the initial concentration at 1.0, 5.0, and 50.0 μg/mL, respectively.

The freeze–thaw stabilities of plasma samples containing 1.0, 5.0, and 50.0 μg/mL of doripenem were examined using an equal volume of 1 M MOPS buffer. The mean concentrations (±S.D.;

Table 3
Recovery study to examine the influence of variations in the protein contents of frozen–thawed plasma and peritoneal fluid samples

Plasma or peritoneal fluid	Recovery (n = 6) (mean ± S.D.) (%)	Peak height ratio of doripenem to I.S. (mean ± S.D.)	Concentration found (mean ± S.D.) (μg/mL)	Albumin (g/dL)	Total protein (g/dL)
Control plasma	92.8 ± 0.7	10.63 ± 0.10	25.10 ± 0.24	4.6	7.7
Control plasma diluted by 50%	94.7 ± 0.2	10.53 ± 0.02	24.85 ± 0.04	2.3	3.9
Control plasma diluted by 25%	96.7 ± 0.3	10.51 ± 0.07	24.81 ± 0.16	1.2	1.9
Plasma A	92.5 ± 0.5	10.54 ± 0.06	24.89 ± 0.14	4.6	7.1
Plasma B	92.4 ± 0.3	10.53 ± 0.10	24.86 ± 0.25	4.3	7.1
Plasma C	91.9 ± 0.4	10.60 ± 0.05	25.04 ± 0.13	4.4	7.2
Plasma D	92.4 ± 1.7	10.55 ± 0.10	24.90 ± 0.23	4.8	7.9
Plasma E	92.0 ± 0.6	10.57 ± 0.05	24.95 ± 0.11	4.7	7.2
Control peritoneal fluid	90.7 ± 0.5	10.54 ± 0.18	25.01 ± 0.43	1.1	2.1
Control peritoneal fluid diluted by 50%	94.6 ± 1.3	10.59 ± 0.03	25.13 ± 0.08	0.6	1.1
Control peritoneal fluid diluted by 25%	96.4 ± 0.4	10.47 ± 0.03	24.85 ± 0.08	0.3	0.5
Peritoneal fluid F	92.2 ± 0.4	10.54 ± 0.05	25.02 ± 0.11	1.4	2.5
Peritoneal fluid G	92.6 ± 0.5	10.48 ± 0.04	24.86 ± 0.09	2.2	3.7
Peritoneal fluid H	94.3 ± 0.3	10.57 ± 0.07	25.07 ± 0.16	0.9	1.8
Peritoneal fluid I	91.9 ± 0.9	10.64 ± 0.04	25.25 ± 0.11	1.2	2.4
Peritoneal fluid J	90.0 ± 0.2	10.66 ± 0.10	25.31 ± 0.23	2.0	4.9

The concentration of doripenem was 25 μg/mL.

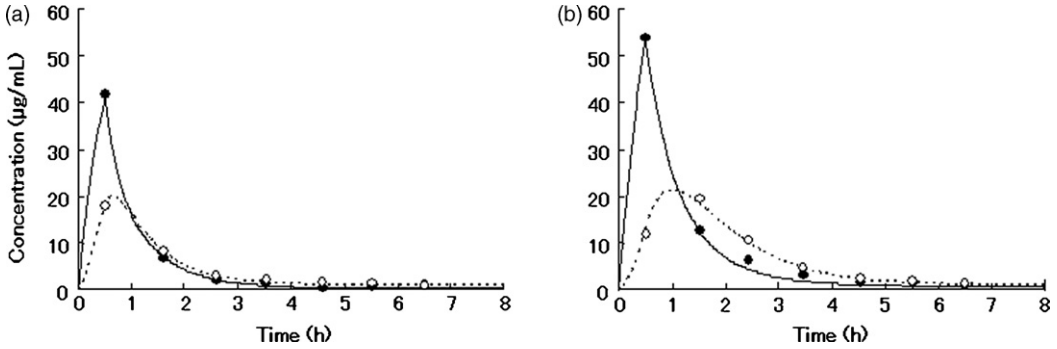

Fig. 4. Time course of plasma and peritoneal fluid doripenem concentrations in two patients who received a 0.5 h infusion of doripenem (500 mg) every 8 h. (a) Patient A; (b) patient B. Symbols: measured data; lines: change in doripenem concentration fitted to three-compartment model; solid circle and solid line: plasma doripenem concentrations; open circle and dotted line: peritoneal fluid doripenem concentrations.

Table 4
Patient information and pharmacokinetic data from the patients who received a 0.5 h infusion of doripenem (500 mg)

Patient	Weight (kg)	C_{max} in plasma (μg/mL)	C_{max} in Peritoneal fluid (μg/mL)	T > MIC (%)[a]	
				Plasma MIC = 4 μg/mL	Peritoneal fluid MIC = 4 μg/mL
A	52	41.0	20.0	31	28
B	61	53.9	21.0	35	46

[a] Predicted T > MIC (% of 24 h) when doripenem (500 mg) was administered every 8 h.

n = 3 for each) after three freeze–thaw cycles were 102.8 ± 0.3%, 102.4 ± 1.7%, and 104.4 ± 1.8% for the initial concentrations of 1.0, 5.0, and 50.0 μg/mL, respectively. The freeze–thaw stabilities of peritoneal fluid samples containing 1.0, 5.0, and 50.0 μg/mL of doripenem were also examined, using an equal volume of 1 M MOPS buffer. The mean concentrations (±S.D.; n = 3 for each) after three freeze–thaw cycles were 100.2 ± 2.3%, 101.0 ± 1.0%, and 99.7 ± 0.4% for the initial concentrations of 1.0, 5.0, and 50.0 μg/mL, respectively.

3.8. Application to peritoneal pharmacokinetic studies in patients

Fig. 4 shows the results from peritoneal pharmacokinetic studies in two patients. Although the simulated peak concentrations of doripenem in plasma were 41.0 and 53.9 μg/mL for patients A and B, respectively, the corresponding peak concentrations in peritoneal fluid of doripenem were 20.0 and 21.0 μg/mL (Table 4). However, the T > MIC in peritoneal fluid at the MIC of 4 μg/mL were equivalent or somewhat larger than those in plasma. The peritoneal pharmacokinetic studies on these two patients revealed that intravenous doripenem penetrated the peritoneal fluid rapidly and extensively. The drug-exposure times in peritoneal fluid were greater than or equal to those in plasma (Table 4), maintaining over 20% of the T > MIC required for bactericidal effects of doripenem [12].

4. Discussion

The LOD and LOQ values of the newly developed method were 0.02 and 0.05 μg/mL, respectively. Therefore, the newly developed method showed a 10-fold enhancement of sensitivity compared to the method of Sutherland and Nicolau, which has an LOQ of 0.5 μg/mL [6]. Moreover, both the sample preparation time (10 min of centrifugation) and HPLC run-time (10 min) of the new method are much shorter than the corresponding times in the earlier method [6].

Doripenem bound to plasma or peritoneal fluid protein cannot be separated by ultrafiltration. However, this may have minimal influence on the ultrafiltration method, since the percentage of doripenem bound to plasma protein is small (8.9%) [5]. To confirm this, we examined the influence of variation in the plasma or peritoneal fluid protein content on recovery (Table 3). When control plasma or peritoneal fluid (frozen–thawed samples) diluted 1:2 and 1:4 with water were compared, the recovery tended to increase at lower concentrations of plasma or peritoneal fluid. However, the peak height ratios of doripenem to IS were almost the same. Therefore, the concentrations detected were not influenced by the albumin concentration or total protein concentration of plasma or peritoneal fluid, although the possibility cannot be excluded that the protein binding of doripenem was lower in frozen-and-thawed samples than in freshly collected samples.

By including ultrafiltration to remove plasma and peritoneal fluid proteins more easily and rapidly as compared with conventional techniques, the method enabled us to obtain these results from the two patients within 1 h after final sampling, with time-management analysis and individualized real-time medical treatment. After a 10 min centrifugation, the filtrates can be applied to HPLC, which requires only a few minutes and no special techniques. We believe that the newly developed method will be very useful in the clinical setting as well as for pharmacokinetic–pharmacodynamic studies.

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