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RESEARCH PAPER

Measurement of meropenem concentration in different human biological fluids by ultra-performance liquid chromatography-tandem mass spectrometry

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Abstract Meropenem is a broad-spectrum antibiotic, often used for the empirical treatment of infections in critically ill patients with acute kidney injury. Meropenem has clinically insignificant protein binding and, as a carbapenem antibiotic, shows time-dependent bacterial killing, meaning that the unbound or free antibiotic concentration in blood should be maintained above the minimal inhibitory concentration of the pathogen for at least 40 % of the dosing interval. We developed and validated simple chromatographic methods by ultra-performance liquid chromatography-tandem mass spectrometry to measure plasma, filtrate-dialysate, and urine concentrations of meropenem. Chromatographic separation was achieved using an Acquity® UPLC® BEH™ (2.1 × 100 mm id, 1.7 μm) reverse-phase C_{18} column, with a water/acetonitrile linear gradient containing 0.1 % formic acid at a 0.4-mL/min flow rate. Meropenem and its internal standard (ertapenem) were detected by electrospray ionization mass spectrometry in positive ion multiple reaction monitoring mode. The limits of quantification were 0.27, 0.24, and 1.22 mg/L, and linearity was observed between 0.27–150, 0.24–150, and 1.22–2,000 mg/L for plasma, filtrate-dialysate, and urine samples, respectively. Coefficients of variation and relative biases were less than 13.5 and 8.0 % for all biological fluids. Recovery values were greater than 68.3 %. Evaluation of the matrix effect showed ion suppression for meropenem and ertapenem. No carry-over was observed. The validated methods are useful for both therapeutic drug monitoring and pharmacokinetic studies. It could be applied to daily clinical laboratory practice to measure the concentration of meropenem in plasma, filtrate-dialysate, and urine.

Keywords Filtrate-dialysate · Ertapenem · Meropenem · Plasma · Therapeutic drug monitoring · UPLC-MS/MS · Urine

Abbreviations

δ_r	Bias
CLSI	Clinical and Laboratory Standards Institute
CRRT	Continuous renal replacement therapy
CV	Coefficient of variation
CVVHDF	Continuous venovenous hemodiafiltration
EMA	European Medicines Agency
ESI	Electrospray ionization
ETM	Ertapenem
$fT>MIC$	Free-drug time above bacteria minimal inhibitory concentration
HPLC	High-performance liquid chromatography
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
LC	Liquid chromatography
LLOD	Lower limit of detection
LLOQ	Lower limit of quantification

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MEM	Meropenem
MIC	Minimal inhibitory concentration
MRM	Multiple reaction monitoring
MS	Mass spectrometer
MS/MS	Tandem mass spectrometry
m/z	Mass-to-charge
PD	Percent deviation of the nominal concentration
PK/PD	Pharmacokinetic/pharmacodynamic
QC	Quality control
S/N	Signal-to-noise
TDM	Therapeutic drug monitoring
ULOQ	Upper limit of quantification
UPLC	Ultra-performance liquid chromatography
UV	Ultraviolet

Introduction

Meropenem ((4*R*,5*S*,6*S*)-3-[(3*S*,5*S*)-5-(dimethylcarbamoyl)pyrrolidin-3-yl]sulfanyl-6-[(1*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid; MEM) (Fig. 1A) is a broad-spectrum β-lactam antibiotic widely used in the empiric treatment of critically ill septic patients receiving continuous renal replacement therapies (CRRT) [1]. Because of its time-dependent pattern of activity, therapeutic drug monitoring (TDM) practices are based on pharmacokinetic/pharmacodynamic (PK/PD) criteria although today it is not clear which PK/PD target is associated with the highest probability of reaching clinical cure [1]. While some studies suggest a target of 40 to 70 % free-drug time above bacteria minimal inhibitory concentration (*f*T>MIC), others recommend increasing the *f*T>MIC to 100 % or even maintaining the MEM concentration four to five times above the MIC for the entire dosage [2, 3]. Meropenem has clinically insignificant protein binding, and dose adjustment is required in renal failure but not in hepatic impairment or geriatric patients [1–3].

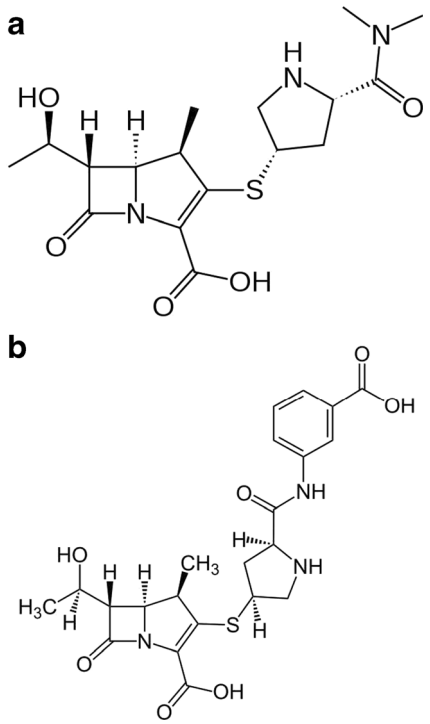

Fig. 1 Molecular structure of **a** meropenem and **b** ertapenem (internal standard)

High-performance liquid chromatography (HPLC) methods for the measurement of MEM in different human plasma, filtrate-dialysate, or urine concentrations using ultraviolet (UV) detection have been previously described [4–10]. However, HPLC-UV techniques are less selective than HPLC-tandem mass spectrometry (MS/MS). In particular, in critically ill polymedicated patients, the measurement of MEM concentration using HPLC-UV could be incorrect because of the risk of co-elution of unspecified endogenous or exogenous compounds with similar chemical structure.

Different methods for the measurement of MEM concentration in human plasma based on HPLC-MS/MS have been published [11–15], but to our knowledge, none of them has been used to measure MEM concentrations in other human biological fluids such as the filtrate-dialysate and urine. The aim of this work was to develop and validate ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) methods for the measurement of MEM concentration in human plasma, filtrate-dialysate, and urine and to apply these measurements in a pharmacokinetic study of meropenem in critically ill patients with sepsis and acute renal failure and receiving continuous renal replacement therapy.

Material and methods

Chemicals and reagents

Meropenem was donated by Fresenius Kabi AG (Bad Homburg, Germany) and ertapenem ((4*R*,5*S*,6*S*)-3-[(3*S*,5*S*)-5-[(3-carboxyphenyl)carbamoyl]pyrrolidin-3-yl]sulfanyl-6-[(1*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid; ETM) (Fig. 1B) by Merck Sharp & Dohme Corp. (Whitehouse Station, NJ, USA). LC-MS-grade formic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). LC-MS-grade acetonitrile and water were supplied by Merck Millipore Group (Darmstadt, Germany).

Calibration standards, quality control samples, and internal standard

Separate stock solutions containing MEM were prepared by dissolving the required amount of MEM (calculated as free

base) in 100 mL of water. Drug-free human plasma, filtrate-dialysate, and urine were obtained from pools of different patients and were used as a biological matrix for calibration standards and quality controls (QC).

Various quantities of stock solution were diluted with water, resulting in eight working standards over the concentration range from the lower limit of quantification (LLOQ) to the upper limit of quantification (ULOQ). Calibration standards (0.0, 5.0, 10.0, 25.0, 50.0, 75.0, 100.0, and 150.0 mg/L for plasma and filtrate-dialysate and 0, 25, 100, 250, 500, 1,000, 1,500, and 2,000 mg/L for urine) were prepared by diluting one part of the working standard with nine parts of the drug-free plasma, filtrate-dialysate, or urine. QC samples were similarly prepared using a separate stock solution. Plasma and filtrate-dialysate QC were prepared at concentrations of 1.0, 30.0, and 90.0 mg/L and urine QC at concentrations of 5.0, 225, and 1,250 mg/L. The calibration standards and the QC samples were stored light-protected for up to 6 months at −75±3 °C as 100-μL aliquots in 1.5-mL polypropylene microcentrifuge tubes and thawed on the day of analysis.

An aqueous preparation containing ETM 2.0 g/L was used as stock solution of internal standard. The working solution (30.0 mg/L ETM) was obtained by diluting the stock solution in acetonitrile.

Sample preparation

Fifty microliters of calibration standards, either QC or plasma, filtrate-dialysate, or urine samples, was transferred to 1.5-mL polypropylene microcentrifuge tubes, and 200 μL of ETM working solution was added to the mixture. After vortexing for 2 min, the tubes were centrifuged for 10 min at 13,000*g* at room temperature. After centrifugation, 100 μL of the supernatant was diluted with 900 μL of 0.1 % (*v*/*v*) aqueous formic acid, vortexed for 5 s, transferred into UPLC vials, and placed in the autosampler ready for injection.

Instrumentation

Analyses were conducted using an Acquity® UPLC® integrated measurement system (Waters, Milford, MA, USA) consisting of a thermostatic autosampler, a binary solvent delivery manager, and a column over a thermostated compartment. Separation was performed on an Acquity® UPLC® BEH™ C_{18} reverse-phase column, 2.1×100 mm, packed with 1.7-μm particles (Waters, Milford, MA, USA). The column chamber was held at a temperature of 40 °C.

A gradient mobile phase was applied with solvent A containing 0.1 % (*v*/*v*) formic acid in water and solvent B containing 0.1 % (*v*/*v*) formic acid in acetonitrile. The mobile phase flow rate was maintained at 0.4 mL/min. From 0.0 to 0.7 min, isocratic conditions were run with 95 % A. Solvent A was decreased from 95 to 45 % in the time range from 0.7 to 1.8 min (linear gradient). Re-equilibration was performed from 1.4 to 3.0 min at 95 % A. The injection volume was 10 μL in a 50-μL loop (partial loop with needle overfill injection mode), and the autosampler was held at a temperature of 4±1 °C.

Detection was carried out using an Acquity® TQD® tandem-quadrupole MS equipped with a Z-spray electrospray ionization (ESI) source (Waters, Milford, MA, USA) operating in positive mode. Nitrogen was used as the nebulizing and desolvation gas, and argon was used as collision gas. The optimized MS settings employed for MEM and ETM were capillary potential 1.2 kV, extractor voltage 3 V, RF lens voltage 0.1 V, source temperature 140 °C, desolvation temperature 350 °C, desolvation gas flow rate 800 L/h, collision gas flow 0.20 mL/min, cone voltage 25 V for MEM and ETM, and collision energy 15 eV for MEM and ETM. MEM and ETM were detected in multiple reaction monitoring (MRM) mode using mass-to-charge (*m*/*z*) transition of 384.2→141.0 and 476.2→346.0, respectively. The scan dwell time was set at 40 ms for every channel.

Validation procedure

Validation was performed according to the European Medicines Agency (EMA) and Clinical and Laboratory Standards Institute (CLSI)-International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) guidelines [16–18].

Calibration

Eight-level calibration standards containing MEM were processed daily before MEM quantification. Integration of smoothed peak areas and calculation of MEM concentration were performed with TargetLynx™ v 4.1 software (Waters, Milford, MA, USA). According to the EMA guideline [16], calculated concentrations of the calibration standards were all within ±15 % of the nominal value, except for the LLOQ for which it was within ±20 %.

The calibration curves were generated by linear fit of the MEM/ETM standard response ratio versus MEM concentration ($1/X$ weighting; excluding the option to force through the point of origin).

Selectivity

Thirteen different batches of plasma, filtrate-dialysate, and urine from patients not treated with MEM or ETM but receiving, in combination or alone, other drugs such as penicillins (amoxicillin and piperacillin), cephalosporins (cefuroxime, cefepime, and cetriaxone), and β-lactamase inhibitors (clavulanic acid and tazobactam) were used. Three patients receiving amoxicillin/clavulanic acid, three piperacillin/

tazobactam, three ceftriaxone, three cefepime, and one cefuroxime had antibiotic concentrations maintained above the minimal inhibitory concentration of the pathogen.

According to the EMA guideline [16], the absence of interfering components is accepted when the peak area response of the interfering peak at the retention time of analyte (MEM) is less than 20 % of the LLOQ for the analyte (MEM) and 5 % for the internal standard (ETM).

Limit of detection and limit of quantification

The CLSI-IFCC C50-A guideline [17] defines the LLOD as the lowest concentration where the signal-to-noise (*S*/*N*) ratio is three or more and the LLOQ as the lowest concentration at which the *S*/*N* ratio is 10 or more and that could be estimated with an acceptable inter-day imprecision (coefficient of variation of ≤20 %).

To estimate the LLOD and the LLOQ, the calibrator level 1 (5 mg/L for plasma and filtrate-dialysate samples and 25 mg/L for urine samples) was diluted 5-fold, 10-fold, and 20-fold with its respective drug-free plasma, filtrate-dialysate, or urine pool, and each concentration was processed repeatedly 20 times in 1 day and in a single series per day.

Carry-over

In accordance to the EMA guideline [16], carry-over was assessed by injecting a blank sample (0.0 mg/L) after a high-concentration sample at the upper limit of quantification (150 mg/L for plasma and filtrate-dialysate samples and 2,000 mg/L for urine samples). Carry-over is accepted if the peak area response in the blank sample obtained after measurement of the high-concentration sample is not greater than 20 % of the analyte (MEM) peak area response at the LLOQ and 5 % the peak area response of the internal standard (ETM).

Imprecision and bias

Four samples, three QC and one LLOQ, were used to estimate intra-day and inter-day imprecision and bias according to the following equations:

$$\mathrm{CV}(\%) = \left(s/\bar{x} \right) \cdot 100 \quad \delta_r(\%) = \left[\left(\bar{x} - \mu \right) / \mu \right] \cdot 100$$

where CV, s, $\bar{x}$, δ_r, and μ are coefficient of variation, standard deviation, mean, relative bias, and the conventional value, respectively. The conventional value of the control samples was assigned by weighting procedures.

For intra- and inter-day imprecision and bias, 20 aliquots of each concentration were tested repeatedly in 1 day and in a single series per day, for 45 days, respectively. Coefficient of variation and δ_r results were analyzed following the EMA acceptance criteria (15 % for QC materials and 20 % for LLOQ).

Measurement interval (linearity)

According to the EMA and the CLSI EP6-A guidelines [16, 18], to calculate the measurement interval, the highest concentration calibrator (150 mg/L for plasma and filtrate-dialysate samples and 2,000 mg/L for urine samples) was diluted with the lowest concentration calibrator (0.0 mg/L) at ratios of 4:0, 3:1, 2:2, 1:3, and 0:4 to achieve theoretical concentrations of 150.0, 112.5, 75.0, 37.5, and 0.0 mg/L for plasma and filtrate-dialysate samples and 2,000, 1,500, 1,000, 500, and 0.0 mg/L for urine samples, respectively. According to the EMA guideline [16], each dilution was measured in triplicate, and imprecision and bias should be ±15 %.

The measured values were plotted on the *y*-axis versus the expected or known values on the *x*-axis. The measured values were considered to be linear following criteria from the CLSI document EP6-A [18], as assessed in the Analyse-it® statistical software (Analyse-it Software, Ltd., Leeds, UK).

Recovery

For the recovery study, several MEM-spiked samples were prepared (1.0, 30.0, and 90.0 mg/L for plasma and filtrate-dialysate samples and 5.0, 225, and 1,250 mg/L for urine samples). Recovery was calculated as the mean ratio between the peak area response of six replicates of these samples and the corresponding peak area response of equivalent neat samples. The recovery of ETM was similarly studied. According to the CLSI-IFCC C50-A guideline [17], the variation in recovery between all concentrations should be less than 15 %.

Matrix effect

According to the EMA guideline [16] and Viswanathan et al. [19], the quantitative measure of the matrix effect can be termed as the matrix factor and defined as the ratio of the peak area response in the presence of the matrix (measured by analyzing a blank matrix spiked after extraction with analyte) to the peak area response in the absence of the matrix (pure solution of analyte):

$$\text{Matrix factor} = \frac{\text{Peak area response in the presence of matrix components}}{\text{Peak area response in the absence of matrix components}}$$

A matrix factor greater than 1 may be due to ion enhancement, and that less than 1 may be due to ion suppression. Similarly, the internal standard can also experience ion enhancement or ion suppression.

To take into account the matrix effects of the internal standard (in our case, ETM), an ETM-normalized matrix

factor was calculated by dividing the matrix factor of the MEM by the matrix factor of the ETM. The ETM-normalized matrix factor was calculated in six different lots of matrix plasma, filtrate-dialysate, and urine at three different concentrations (1.0, 30.0, and 90.0 mg/L for plasma and filtrate-dialysate samples and 5.0, 225, and 1,250 mg/L for urine samples) to determine the variability of the matrix effect in samples from different individuals.

According to EMA and CLSI-IFCC C50-A guidelines, the variability in matrix effect as measured by the coefficient of variation should be less than 15 % and the variation in matrix effect between all concentrations should be less than 15 %.

Stability study

Stability studies included stock solution stabilities of MEM and ETM, extracted samples in-autosampler stability, and short- and long-term stabilities for MEM.

To evaluate the effect of refrigeration, the peak area response of the stock solutions refrigerated at 5±3 °C for 2, 5, and 7 days were compared with fresh stock at room temperature. The stability of samples in the autosampler was tested, reinjecting them after at 6, 12, and 24 h of storage at 4±1 °C. To evaluate short-term stability, the aliquots for QC were first stored at 5±3 °C for 2, 5, and 7 days and then equilibrated to room temperature and extracted and tested against their fresh counterparts. For long-term stability evaluation, the aliquots for QC were first frozen at −75±3 °C for 6 months and then thawed before extraction and tested against fresh calibration and QC samples.

All stability exercises were carried out using ten replicates of QC samples against fresh calibration standards, and the results were compared with the fresh QC samples. The European Medicines Agency [16] defines stable samples as those mantaining a mean concentration at each level within ±15 % of the nominal concentration.

Application to biological samples

The UPLC-MS/MS methods described here were developed to measure MEM concentrations in human plasma, filtrate-dialysate, and urine for pharmacokinetic investigations, from critically ill patients with sepsis and acute renal failure and receiving continuous renal replacement therapy, and they are currently applied in a research protocol. This study was carried out according to the principles of the Declaration of Helsinki and was approved by the local Ethics Committee of Bellvitge University Hospital. Written informed consent according to local practice was obtained for every patient.

Meropenem administration

A standard dose of Meropenem Kabi® (Fresenius Kabi AG, Bad Homburg, Germany), 1,000 mg, was given to patients every 8 h as a 30-min intravenous perfusion in accordance to local department guidelines.

Renal replacement therapy

Continuous venovenous hemodiafiltration (CVVHDF) was performed in all the patients using the set oXiris™ (Hospal, Lyon, France) in conjunction with the Prismaflex eXeed II™ system (Hospal, Lyon, France). Vascular access was obtained via the subclavian, internal jugular, or femoral vein, using a 13-F dual-lumen catheter. Blood flow was maintained between 200 and 250 mL/min, and ultrafiltrate flow was 2,000 mL/h. The filter was changed in accordance with local department guidelines.

Sample collection

Pharmacokinetic sampling occurred during 24 h at steady state. Patients were initiated on CVVHDF and MEM at least 24 h prior to the sampling period (meaning that steady state was achieved). The sampling period was initiated 1 or 2 h after the filter replacement. Approximately, 2 mL of blood was collected in a lithium heparin tube (Vacuette, Kremsmünster, Austria) pre- and postfilter, predose, and at 0.50, 0.75, 1, 2, 4, 8, 16, and 24 h and immediately refrigerated at 4 °C. Filtrate-dialysate samples were collected at the same time points and conditions. Blood and filtrate-dialysate samples were centrifuged at 2,000*g* for 10 min at 4±1 °C, aliquoted, and stored at −75±3 °C until analysis. Urine samples were collected at 24 h, immediately refrigerated at 4 °C, centrifuged at 400*g* for 10 min at 4±1 °C, and kept at −75±3 °C until analysis.

Results

Under the chromatographic conditions described above for UPLC-MS/MS methods, the retention times for MEM and ETM were 1.05 and 1.09 min, respectively, for all human biological fluids. Typical chromatograms are shown in Fig. 2. The UPLC-MS/MS run time was 3.0 min, including the time necessary for the solvent gradient to return to baseline conditions before the next injection.

Selectivity

No interfering peaks were present in any plasma, filtrate-dialysate, or urine sample from patients receiving other antibiotics. The peak area responses observed in all plasma,

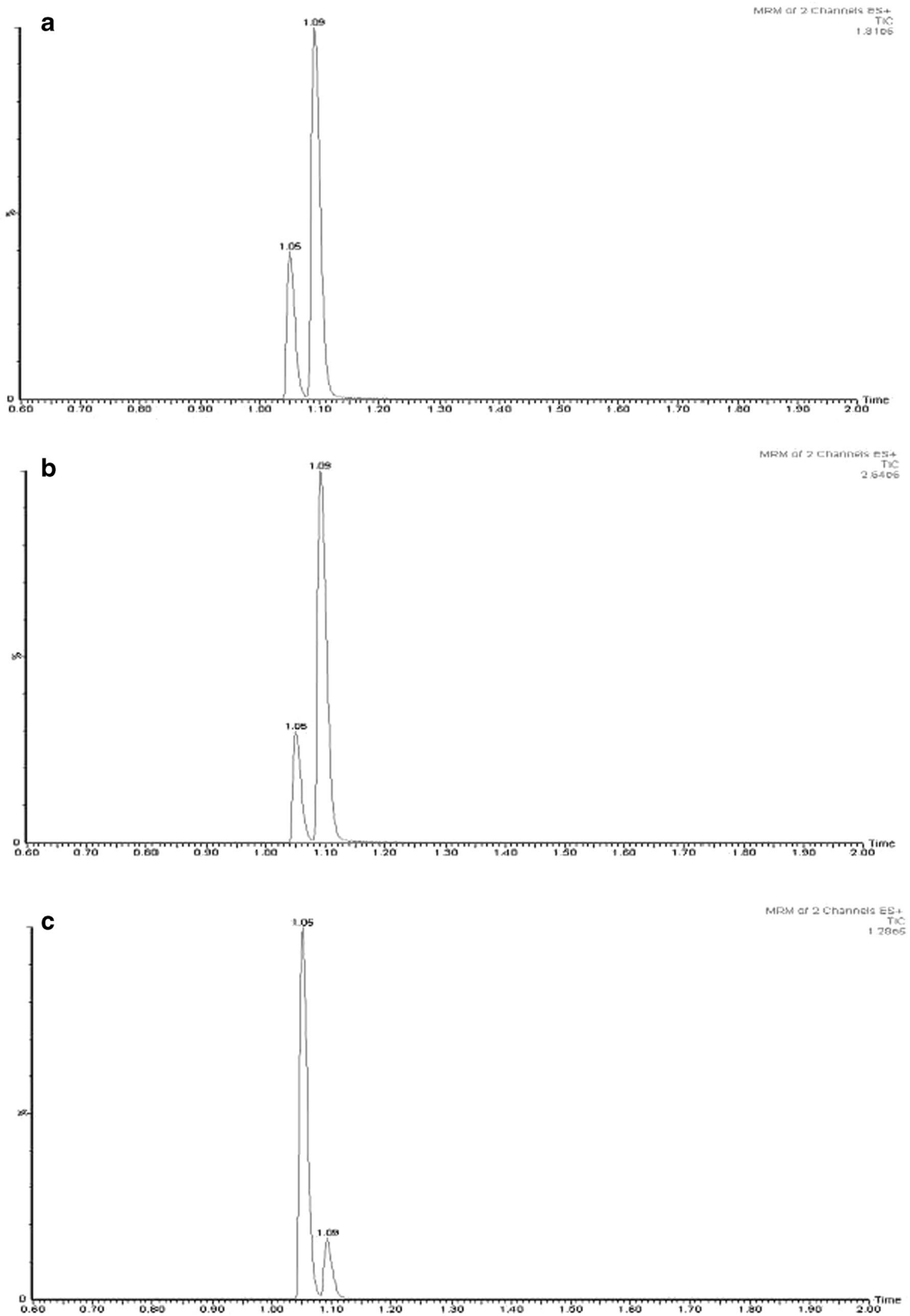

Fig. 2 A representative MRM total ion chromatogram obtained from human **a** plasma patient sample at a concentration of 7.97 mg/L, **b** filtrate-dialysate patient sample at a concentration of 4,78 mg/L, and **c** urine patient sample at a concentration of 1,502 mg/L

filtrate-dialysate, and urine batches at MEM retention time were ≤3.6 % of the LLOQ of MEM, being less than 0.9 % at the ETM retention time.

Limit of detection and limit of quantification

The LLOD were 0.09 mg/L (*S*/*N* ratio of 3.8), 0.07 mg/L (*S*/*N* ratio of 4.4), and 0.29 mg/L (*S*/*N* ratio of 4.2) for plasma, filtrate-dialysate, and urine samples, respectively. The LLOQ were 0.27 mg/L (*S*/*N* ratio of 11.5, *CV* of 17.2 %), 0.24 mg/L (*S*/*N* ratio of 13.2, *CV* of 15.4 %), and 1.22 mg/L (*S*/*N* ratio of 10.5, *CV* of 16.4 %) for plasma, filtrate-dialysate, and urine samples, respectively.

Carry-over

Peak area responses observed in the blank samples after measurement of the high-concentration samples were 9.4, 8.1, and 8.7 % of the MEM peak area response at the LLOQ for plasma, filtrate-dialysate, and urine samples, respectively. On the other hand, peak area responses were 2.7, 1.9, and 2.6 % of the peak area response of the ETM.

Imprecision and bias

Data for intra-day and inter-day imprecision and relative bias are summarized in Table 1. Imprecision and relative bias were lower than the maximum permissible requirements for these metrological characteristics (15 % for quality control materials and 20 % for LLOQ).

Measurement interval (linearity)

The measurement interval was found linear between 0.27 and 150 mg/L, 0.24 and 150 mg/L, and 1.22 and 2,000 mg/L for plasma, filtrate-dialysate, and urine samples, respectively. The resulting mean measured values of each sample were compared to predicted concentrations and yielded a linear regression of $y=1.086x-4.883$ ($r^2=0.9985$), $y=1.074x-2.225$ ($r^2=0.9995$), and $y=1.034x-11.875$ ($r^2=0.9923$) for plasma, filtrate-dialysate, and urine samples, respectively. Dilution integrity of the samples achieved acceptance criteria for imprecision and bias (15 %).

Recovery

The mean recoveries for MEM and ETM from plasma, filtrate-dialysate, and urine samples were greater than 68.3 % (Table 2). The variations in recovery between all concentrations (CV) were less than 12.2 and 11.6 % for MEM and ETM for all cases (Table 2).

Table 1 Intra-day and inter-day imprecision and bias values obtained in UPLC-MS/MS for meropenem concentration in plasma, filtrate-dialysate, and urine QC samples

Biological fluid/theoretical concentration in mg/L (sample type)	Intra-day (n=20)			Inter-day (n=20)		
	$\bar{x}\pm s$ (mg/L)	CV (%)	δ_r (%)	$\bar{x}\pm s$ (mg/L)	CV (%)	δ_r (%)
Plasma						
0.25 (LLOQ)	0.25±0.041	16.4	0.0	0.27±0.047	17.2	8.0
1.0 (QC1)	1.10±0.13	11.9	10.0	0.96±0.15	13.5	−4.0
30.0 (QC2)	31.1±2.12	6.8	3.7	31.4±2.35	7.5	4.6
90.0 (QC3)	88.1±1.72	1.9	−2.1	86.7±3.98	4.6	−3.6
Filtrate-dialysate						
0.25 (LLOQ)	0.23±0.033	14.3	−8.0	0.24±0.037	15.4	−4.0
1.0 (QC1)	1.08±0.01	9.2	8.0	1.01±0.11	11.3	1.0
30.0 (QC2)	29.4±1.82	6.2	−2.0	29.1±2.07	7.1	−3.0
90.0 (QC3)	90.5±1.49	1.6	−0.6	92.9±2.97	3.2	3.2
Urine						
1.25 (LLOQ)	1.24±0.18	14.5	−0.8	1.22±0.20	16.4	−2.4
5.0 (QC1)	4.48±0.42	9.4	−10.4	4.91±0.59	12.0	−1.8
225 (QC2)	219±14.2	6.4	−2.7	235±18.3	7.8	4.4
1,250 (QC3)	1,199±28.8	2.4	−4.8	1,301±53.3	4.1	4.1

n number of samples processed, $\bar{x}$ mean value, *s* standard deviation, CV coefficient of variation, δ_r relative bias, *LLOQ* low limit of quantification, *QC1* internal quality control 1, *QC2* internal quality control 2, *QC3* internal quality control 3

Table 2 Recovery values (%) for meropenem and ertapenem (internal standard)

Biological fluid	Sample concentration (mg/L)	Meropenem recovery $\bar{x}\pm s$ (%)	Ertapenem recovery $\bar{x}\pm s$ (%)
Plasma	1.0	74.1±7.1	73.1±7.5
	30.0	77.2±4.9	77.9±5.1
	90.0	81.4±3.5	79.3±3.8
$\bar{x}$ (%)		**77.6**	**76.8**
s (%)		**6.3**	**6.4**
CV (%)		**8.1**	**8.3**
Filtrate-dialysate	1.0	64.5±6.4	66.1±7.2
	30.0	72.2±5.6	72.9±6.4
	90.0	73.3±4.8	76.3±4.1
$\bar{x}$ (%)		**70.0**	**71.7**
s (%)		**7.1**	**7.6**
CV (%)		**10.1**	**10.6**
Urine	5.0	63.3±8.9	66.1±9.5
	225	69.4±6.9	69.8±7.2
	1,250	72.3±5.5	71.3±5.8
$\bar{x}$ (%)		**68.3**	**69.1**
s (%)		**8.3**	**8.0**
CV (%)		**12.2**	**11.6**

$\bar{x}$ mean value, s standard deviation, CV coefficient of variation

Matrix effect

Values for matrix factor, variabilities of matrix effect, and ETM-normalized matrix factor in different lots of plasma, filtrate-dialysate, and urine are summarized in Table 3. Evaluation of the matrix effect showed ion suppression for MEM for all cases. The variation in matrix effect between all concentrations was less than 15 %. As shown in Table 3, the matrix effects were well compensated by the internal standard, ETM.

Stability study

Short-term and in-autosampler stability profiles are shown in Fig. 3. Meropenem concentrations in plasma were stable during storage at 5±3 °C for a period of 5 days with an absolute percent deviation of the nominal concentration (PD) lower than 12.9 %. Instead, MEM concentrations were only stable for 2 days in filtrate-dialysate and urine with an absolute PD between 5.4 to 8.2 % and 6.2 to 9.4 %, respectively. On the other hand, MEM concentrations in plasma were stable in the autosampler at 4±1 °C for 12 h (absolute PD≤12.2 %) and for 6 h in filtrate-dialysate and urine (absolute PD≤13.9 and 14.2 %, respectively) (Fig. 3). Also, MEM concentrations in all biological fluids were stable at −75±3 °C for at least 6 months (absolute PD≤7.2 %). Stock solutions of MEM and ETM stored at 5±3 °C were stable for 5 days (absolute PD of 13.2 and 11.4 %, respectively).

Percent deviations were in all cases negative, indicating a loss of MEM or ETM concentration with regard to the nominal value, i.e., a decomposition or degradation of MEM or ETM occurred.

Clinical application

Meropenem concentration values in human plasma, filtrate-dialysate, and urine are being used for developing a population PK/PD model of MEM in critically ill septic patients receiving CRRT [7, 20]. This model would be used in clinical practice to ensure that MEM dosage achieves selected PK/PD criteria. The time profiles of MEM plasma and filtrate-dialysate concentrations during an 8-h interval of three typical patients receiving 1,000 mg of MEM every 8 h are displayed in Fig. 4. Meropenem plasma and filtrate-dialysate concentrations remained above a MIC of 4 mg/L (breakpoint of sensibility) during 100 and 92.6 % of the dosing interval, respectively. If the selected target is four times the MIC, MEM concentration remains above it only 74.8 % for plasma and 40.7 % for filtrate-dialysate of the dosing interval. Meropenem urine concentrations were 947, 1,108, and 1,157 mg/L for the patients shown in Fig. 4 (patients 1, 2, and 3, respectively).

Pharmacokinetic/pharmacodynamic modeling can guide MEM dosage optimization, thus increasing the clinical effectiveness.

Discussion

UPLC-MS/MS methods were developed and validated for the measurement of MEM concentrations in human plasma, filtrate-dialysate, and urine and are being currently applied in a research protocol. These methods could support the therapeutic drug monitoring of MEM in different patients and biological fluids, particularly in critically ill patients with acute kidney injury with sepsis receiving continuous renal replacement therapy. Intrinsic characteristics of the UPLC-MS/MS technique let us achieve similar or shorter retention times than those reached using other methods [11–15].

Method development

Various combinations of reverse-phase UPLC columns and mobile phase were performed to achieve a good resolution and symmetric peaks, a high response, a short retention time, and better peak shape. It was found that the use of a Bridged Ethyl Hybrid UPLC column (Acquity® UPLC® BEH™ C_{18} reverse-phase column, 2.1×100 mm, 1.7 μm) in combination

Table 3 Matrix factor and ETM-normalized matrix factor for meropenem in different lots of plasma, filtrate-dialysate, and urine

Matrix lot	Matrix factor (%)			ETM-normalized matrix factor (%)		
	1.0 mg/L	30.0 mg/L	90.0 mg/L	1.0 mg/L	30.0 mg/L	90.0 mg/L
Plasma 1	65.2	59.9	57.9	101.9	100.2	99.9
Plasma 2	52.7	55.5	51.4	98.6	84.0	89.6
Plasma 3	59.6	46.3	44.9	98.1	104.9	100.1
Plasma 4	66.2	64.6	65.5	89.6	98.4	94.3
Plasma 5	71.1	65.4	55.8	111.1	102.0	126.6
Plasma 6	58.2	52.1	50.2	88.1	87.1	85.1
$\bar{x}$ (%)	**62.2**	**57.3**	**54.3**	**97.9**	**96.1**	**97.0**
***s* (%)**	**6.6**	**7.4**	**7.1**	**8.4**	**8.5**	**9.1**
CV (%)	**10.6**	**12.9**	**13.2**	**8.6**	**8.9**	**9.4**
	1.0 mg/L	30.0 mg/L	90.0 mg/L	1.0 mg/L	30.0 mg/L	90.0 mg/L
Filtrate-dialysate 1	61.4	54.5	55.2	111.1	94.5	89.9
Filtrate-dialysate 2	48.4	46.7	44.4	89.6	89.8	88.8
Filtrate-dialysate 3	60.2	55.4	54.5	99.9	100.9	105.1
Filtrate-dialysate 4	70.2	65.3	62.7	86.4	97.2	99.7
Filtrate-dialysate 5	69.7	63.5	61.8	100.1	113.0	110.6
Filtrate-dialysate 6	54.9	50.0	48.9	87.3	84.3	89.1
$\bar{x}$ (%)	**60.8**	**55.9**	**54.6**	**95.7**	**96.6**	**97.2**
***s* (%)**	**8.4**	**7.3**	**7.1**	**9.7**	**9.9**	**9.4**
CV (%)	**13.9**	**13.1**	**13.1**	**10.1**	**10.2**	**9.6**
	5.0 mg/L	225 mg/L	1,250 mg/L	5.0 mg/L	225 mg/L	1,250 mg/L
Urine 1	51.1	50.2	49.9	95.4	98.9	96.6
Urine 2	48.3	45.9	44.4	103.2	86.4	114.2
Urine 3	52.9	48.8	48.1	100.3	112.9	87.5
Urine 4	61.9	60.6	59.8	98.8	97.4	98.2
Urine 5	45.4	50.0	52.2	78.8	84.6	87.7
Urine 6	62.3	61.9	59.2	99.4	104.8	100.0
$\bar{x}$ (%)	**53.7**	**52.9**	**52.3**	**96.0**	**97.5**	**97.4**
***s* (%)**	**7.0**	**6.7**	**6.2**	**8.8**	**10.8**	**9.8**
CV (%)	**13.1**	**12.6**	**11.8**	**9.2**	**11.1**	**10.1**

$\bar{x}$ mean value, *s* standard deviation, *CV* coefficient of variation

with different linear gradient elution modes based on 0.1 % (*v*/*v*) formic acid with water and acetonitrile let us achieve the chromatographic conditions mentioned above. The choice of the monitored ions was made after studying the MS/MS fragmentation pattern of MEM and ETM. Meropenem and ETM were quantified using the MRM mode due to its high-sensitivity data acquisition when the precursor and the product ions are monitored. The first quadrupole selected the protonated ion at *m*/*z* 384.2 and 476.2, respectively. Fractioned ions *m*/*z* 141.0 for MEM and 346.0 for ETM were detected and selected in the third quadrupole.

Although protein precipitation is probably the extraction method most prone to matrix effects, in our evaluation, a protein precipitation with acetonitrile followed by a dilution in mobile phase simplified the extraction procedures published by others [11–13, 15] and provided acceptable recoveries and matrix effect results. The evaluation and the variability of the matrix effect in samples from different individuals are a key point. These two issues are often not properly studied and could compromise the analysis performance. An ideal internal standard should be a structural analog and a stable and labeled compound and should track the analyte during the extraction and compensate for any analyte on the column and any inconsistent response. Due to problems of availability and the high price of stable labeled compounds, a chemical structural analog of MEM, ertapenem, was the first choice for the analysis. In our evaluation of the matrix effect, we observed that the concentration of the three samples assayed showed a steady value, given that the use of

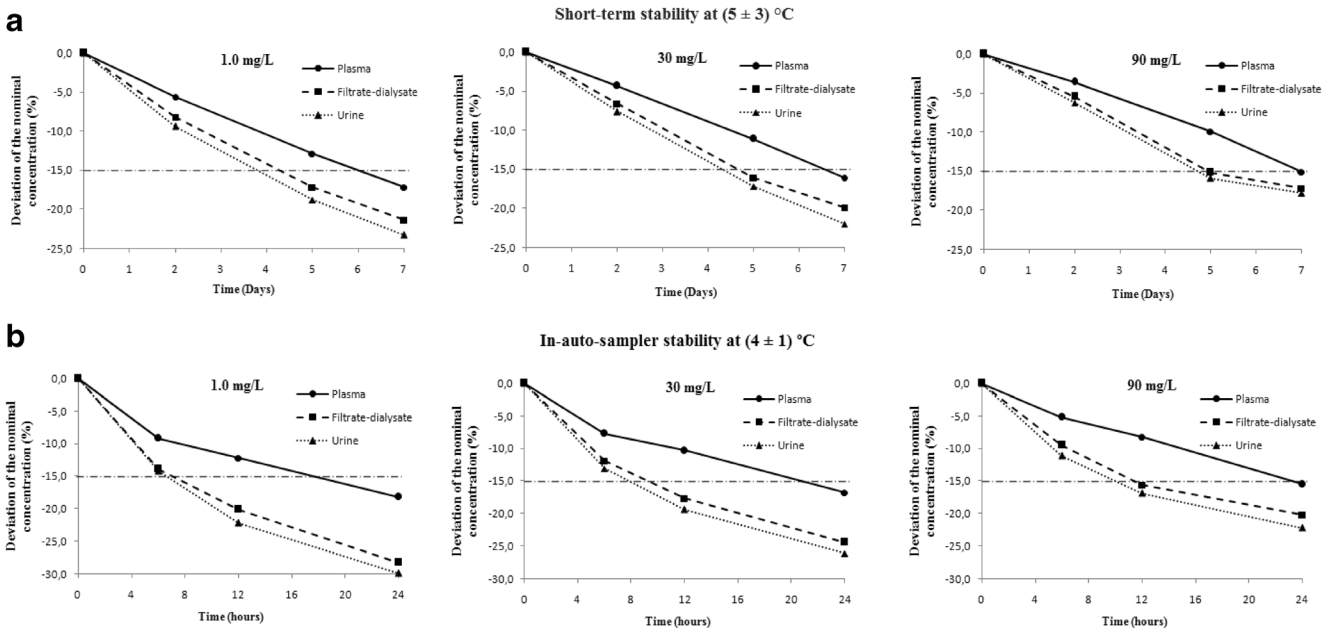

Fig. 3 Short-term stability at 5±3 °C (**a**) and in-autosampler stability at 4±1 °C (**b**) profiles at meropenem concentrations of 1.0, 30, and 90 mg/L

ETM as an internal standard compensates for the ion suppression observed in the MEM. On the other hand, ETM is unlikely to be co-administered with MEM, elutes similarly to the MEM, and will therefore be subject to similar matrix effects. For all these reasons, we considered ETM as a good internal standard.

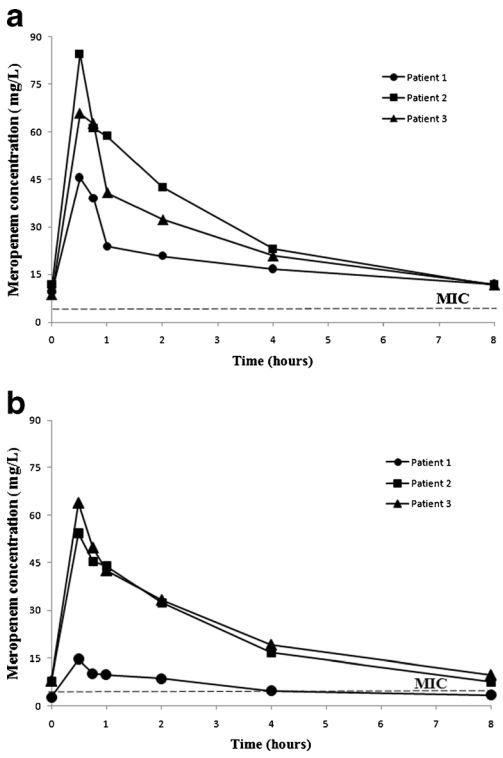

Fig. 4 Concentration-time curve of meropenem in **a** plasma and **b** filtrate-dialysate in three typical patients receiving 1,000 mg of meropenem every 8 h at steady state. Minimal inhibitory concentration (MIC) of susceptible germs=4 mg/L (*dotted line*)

Validation procedure

In the validation procedure, different analytical characteristics and studies were evaluated. No interfering peaks were present in any plasma, filtrate-dialysate, or urine sample from patients receiving other antibiotic drugs indicating that the proposed UPLC-MS/MS methods provide acceptable selectivity. Also, no significant carry-over was observed. Recovery values from all biological samples studied were above 68.3 % for MEM and ETM. The imprecision and bias values obtained for each concentration were found to neither exceed the 15 % of the CV for QC samples nor the 20 % for LLOQ, thus conforming to the EMA criteria [16]. These results indicate that the proposed UPLC-MS/MS methods provide acceptable precision and trueness. UPLC-MS/MS methods presented linearity within the concentration range of 0.27 and 150 mg/L, 0.24 and 150 mg/L, and 1.22 and 2,000.0 mg/L for plasma, filtrate-dialysate, and urine samples, respectively. Linearity and the LLOQ obtained were acceptable, considering the expected concentrations in the pharmacokinetic study.

The poor stability of β-lactams in biological fluids, at room temperature or refrigerated, is well known [9, 11, 21]. We have obtained similar stability results to those published by other authors [9, 11, 21]. For these reasons, precautions should be taken to prevent MEM decomposition in processed samples (i.e., reconstituted extracts in HPLC vials) left at room temperature in the autosampler rack, particularly in filtrate-dialysate and urine. The time during which HPLC vials are stored in the autosampler rack at room temperature should therefore be minimized and the samples placed in the temperature-controlled autosampler just prior to the analysis.

Conclusions

The simple UPLC-MS/MS methods that we have developed and validated for measurement of MEM concentrations in plasma, filtrate-dialysate, and urine could be useful for pharmacokinetic studies in the daily practice of the clinical laboratory. Our work shows different examples of application to MEM monitoring in critically ill patients with acute kidney injury with sepsis and receiving continuous renal replacement therapy.

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