Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 856 (2007) 294–301
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Quantification of meropenem in plasma and cerebrospinal fluid by micellar electrokinetic capillary chromatography and application in bacterial meningitis patients

Yu-Wei Chou [a], Yuan-Han Yang [b], Jin-Hon Chen [b], Chen-Chun Kuo [c], Su-Hwei Chen [a,c,*]

[a] *Graduate Institute of Pharmaceutical Sciences, Kaohsiung Medical University, Kaohsiung 807, Taiwan*
[b] *Department of Neurology, Kaohsiung Medical University Hospital, Kaohsiung 807, Taiwan*
[c] *Department of Pharmacy, Kaohsiung Medical University Hospital, Kaohsiung 807, Taiwan*

Received 19 March 2007; accepted 19 June 2007
Available online 26 June 2007

Abstract

A high-performance micellar electrokinetic capillary chromatography (MEKC) has been demonstrated for the determination of meropenem in human plasma and in cerebrospinal fluid (CSF) and application in meningitis patients after intravenous (IV) administration. Plasma sample was pretreated by means of solid-phase extraction (SPE) on C_{18} cartridge and CSF sample was by direct injection without sample pretreatment, with subsequent quantitation by MEKC. The separation of meropenem was carried out in an untreated fused-silica capillary (40.2 cm × 50 μm I.D., effective length 30 cm) and was performed at 25 °C using a background electrolyte consisting of Tris buffer (40 mM, pH 8.0) solution with sodium dodecyl sulfate (SDS) as the running buffer and on-column detection at 300 nm. Several parameters affecting the separation and sensitivity of the drug were studied, including pH, the concentrations of Tris buffer and surfactant. Using cefotaxime as an internal standard (IS), the linear ranges of the method for the determination of meropenem in plasma and in CSF were all over 0.5–50 μg/mL; the detection limits (signal-to-noise ratio = 3) of meropenem in plasma and in CSF were 0.2 μg/mL and 0.3 μg/mL, respectively.

Keywords: Meropenem; Plasma and cerebrospinal fluid; MEKC; Bacterial meningitis

1. Introduction

Severe brain infections require immediate administration of antibiotics that effectively combat prevalent pathogens. In some cases, empirical antibiotic therapy fails to kill the causative pathogen. It may be an inadequate choice of antimicrobial agent that enables development of bacterial resistance during antibiotic therapy or impaired penetration of the drug into tissue particularly into the brain and CSF. Meropenem, a carbapenem antibiotic agent, has a broad spectrum with good activity against many Gram-negative rods, including *Pseudomonas aeruginosa*, Gram-positive organisms including some β-lactamase-producing pneumococci, and anaerobes involved in bacterial central nervous system infections [1]. Meropenem has advantages in the Intensive Care Unit (ICU) owing to showing good tolerability at high doses and a low incidence of epileptic seizure with meningitis. Therefore, meropenem is the choice for severe infections caused by susceptible microorganisms that are resistant to other available drugs, and for treatment of mixed aerobic and anaerobic infections where large doses of antibiotics are required [2,3]. Meropenem displays a time-dependency whereby bactericidal activity correlates with the duration that serum drug concentration remains above MIC_{90} (minimum inhibitory concentration required to inhibit the growth of 90% of organisms) for the organism. The MIC_{90} value of meropenem concentration for *Pseudomonas aeruginosa* and penicillin- and cephalosporin-resistant *Streptococcus pneumoniae* are less than 0.5 μg/mL and 1.0 μg/mL [4], respectively. Therefore, the meropenem concentration in CSF would be above the MIC.

On the other hand, meropenem is approximately 80% excreted by the kidney. Preexisting renal insufficiency and higher plasma level are risk factors for renal insufficiency and

* Corresponding author at: School of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan. Tel.: +886 7 3121101x2253; fax: +886 7 3210683.
E-mail address: suhwch@kmu.edu.tw (S.-H. Chen).

1570-0232/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2007.06.015

central nervous system side effects. In an individual with renal diseases, the $t_{1/2}$ of the drug is prolonged and meropenem dosage adjustment is necessary for patients based on creatinine clearance with renal impairment [5,6]. It is very important that an adequate and safe level of the drug is achieved in plasma and in CSF for treatment of severe bacterial infection with meningitis.

Aside from the microbiological method, high-performance liquid chromatographic (HPLC) techniques are most commonly employed in clinical use. In general, bioassays lack specificity, and cannot distinguish multiple antibiotics or active metabolites or degradation products from the parent compound. Studies on HPLC with UV detection [7–15] and mass spectrometry [16] have been published for the determination of meropenem in biological samples. The use of capillary electrophoresis (CE) for the determination of meropenem in plasma and urine has been published. These CE analytical methods developed for determining meropenem in plasma and urine involve pretreatment procedures prior to analysis [17,18] or sample direct injection [19]. Each of these methods has unique advantages and disadvantages with respect to sensitivity precision and simplicity of use. Kitahashi and Furuta [19] reported on the analysis of meropenem in plasma with direct sample injection and then the MEKC separation in which the limit of detection of meropenem was reported to be 2 μg/mL. So far, no CE method with direct injection without sample pretreatment for determination of meropenem in human CSF has been reported.

The aim of the study was not only to develop a MEKC method for quantitation of meropenem in plasma and in CSF, but also to evaluate the time–concentration courses of meropenem for plasma as well as for CSF after IV bolus and continuous IV infusion. In this study, we used SPE with C_{18} cartridges for plasma pretreatment; CSF samples were by direct injection without sample pretreatment, and then MEKC was performed. The concentrations of meropenem in two meningitis patients' plasma and CSF samples after continuous-infusion or IV bolus were evaluated.

2. Experimental procedure

2.1. Instrumentation

The Beckman P/ACE MDQ system (Fullerton, CA, USA) equipped with a photodiode-array detector (PDA) and a liquid-cooling device was used. MEKC was performed in an uncoated fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 40.2 cm (effective length 30 cm) × 50 μm I.D. The temperature of the separation was controlled at 25 °C by immersion of the capillary in a cooling liquid circulating in the cartridge. The sample tray was kept at room temperature. Detection was carried out by the on-column measurement of UV absorption at 300 nm (cathode at the detection side).

2.2. Chemicals and reagents

Meropenem (Fig. 1) was kindly supplied by Sumitomo pharmaceuticals (Osaka, Japan) and cefotaxime, as the IS, was from Sigma (St. Louis, MO, USA). Sodium hydroxide (NaOH), phosphoric acid (H_3PO_4, 85%), tris(hydroxymethyl)aminomethane (Tris), sodium dodecyl sulfate (SDS), methanol and other reagents were of analytical grade from E. Merck (Darmstadt, Germany). Milli-Q treated water (Millipore, Bedford, MA, USA) was used for the preparation of buffer and related drugs.

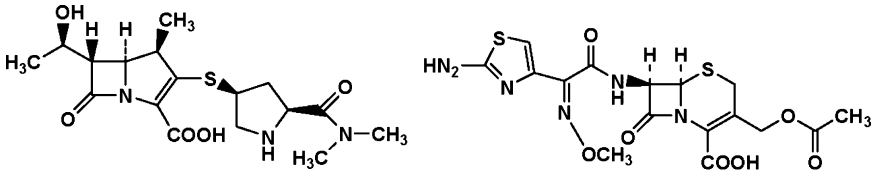
2.3. Preparation of background electrolyte solutions

Solutions of various Tris buffers at different pH levels were prepared by neutralizing Tris solution with H_3PO_4. Solutions of Tris-SDS buffer at various levels of SDS were obtained by dissolving different amounts of SDS in Tris followed by dilution with Tris buffer as a background electrolyte. The final electrolyte solutions containing Tris buffer (40 mM, pH 8.0) with 200 mM SDS were used for plasma and CSF samples analysis. New capillary (50 μm I.D.) conditioning before startup was undertaken with methanol for 10 min, 1 N HCl solution for 10 min, deionized water for 2 min, 1 N NaOH for 10 min, deionized water for 2 min and running electrolyte for 10 min. The conditioning procedure between runs was rinsing with 0.1 N NaOH (4 min), deionized water (2 min), and electrolyte solution (4 min) under positive pressure applied at the injection end. The plasma samples after SPE pretreatment were hydrodynamically injected by 34.5 mbar for 10 s (about 14.8 nL) and CSF samples were electrokinetically direct-injected by 5 kV for 65 s without sample clean-up procedure, then keeping the separation voltage at 15 kV (anode at the injection end) under MEKC mode. The Beckman P/ACE MDQ Microsoft Software system was used for data processing.

2.4. Sample pretreatment and injection modes

2.4.1. For human plasma

Drug-free human plasma samples were obtained from two medicine-free young volunteers (22–24 years old) and plasma



Meropenem Cefotaxime

Fig. 1. Chemical structures of meropenem and cefotaxime (IS).

samples were used singularly as the control. Waters Oasis HLB (60 mg, 3 mL) (Milford, MA, USA) cartridges were used in a Varian (CA, USA) vacuum manifold apparatus for the SPE procedure. The cartridges were activated and conditioned with 1.5 mL of methanol and then 3 mL of water. Amounts of 270 μL of blank plasma spiked with the various concentrations of meropenem and 30 μL of 750 μg/mL of cefotaxime (IS) or 270 μL of patients' plasma and 30 μL of 750 μg/mL of cefotaxime (IS) were loaded onto the cartridges. After loading the plasma sample, the cartridges were washed with 0.5 mL water and dried under vacuum aspiration for 30 s. The analytes were eluted with 0.3 mL of methanol and the cartridge dried under vacuum again for 30 s. The methanol solution was then brought to dryness under freeze evaporator (EYELA UT-80 and EYELA CVE-2000) and redissolved with 30 μL of water and then transferred into a 0.2 mL sample vial that could be placed into the sampler of the CE apparatus. The reconstructed solutions were hydrodynamically injected by 34.5 mbar for 10 s. All meropenem-spiked plasma and CSF samples were stored at −40 °C for stability study.

2.4.2. For human CSF

Drug-free human control CSF samples were obtained from the Department of Neurology, Kaohsiung Medical University Hospital (Kaohsiung Taiwan) and the collected control CSF samples were pooled together. A 90 μL aliquot of human CSF was pipetted into a 1.5 mL Eppendorf vial, and 10 μL aqueous solution containing meropenem and cefotaxime as an IS was added to prepare the final concentrations of meropenem and cefotaxime in CSF were 50 μg/mL and 30 μg/mL, respectively. 1 N hydrochloric acid was added to the CSF samples (v/v, 1:20) to enhance protonation of the cationic drugs and improve sensitivity. The capillary tip was dipped for 6 s into a vial containing water for cleaning. Then a water plug (application of 34.5 mbar for 6 s) was introduced into the capillary. CSF samples were electrokinetically injected at a positive voltage of 5 kV for 65 s (anode at the injection end) and the separation voltage was 15 kV under the MEKC mode.

2.5. Method validation

The calibration graphs over the range of 0.5–50 μg/mL of meropenem with 75 μg/mL or 30 μg/mL of cefotaxime as IS in plasma and in CSF, respectively, were established with the peak area ratio of meropenem to cefotaxime (IS) as ordinate (y) versus the concentration of meropenem in μg/mL as abscissa (x). The precision and accuracy of the method were estimated at low, medium and high concentrations. The intra-day of mean precision was defined by relative standard deviation (RSD) and relative error (RE) from analyses on the same days. The inter-day precision and accuracy were calculated from repeated analyses of identical samples on five consecutive days for these concentrations of meropenem, and expressed as RSD and RE. The limits of detection (LOD) were calculated on the basis of the baseline noise, which was defined as the sample concentration generating a peak of height three times the level of the baseline noise (signal-to-noise ratio of 3).

2.6. Application

The study protocol was approved by the Ethics Committee of the Kaohsiung Medical University Hospital. Two meningitis patients requiring extraventricular drainage due to obstructive hydrocephalus were treated with IV administration of meropenem (Mepem®) at the Department of Neurology ICU. One of the meningitis patients, a female, received IV bolus of 2 g over 20 min every 8 h. Venous blood sample and CSF were withdrawn at 4 h after dosing. Several days later, the patient's infection symptoms had not subsided, therefore, the patient received 2 g meropenem IV administration from IV bolus changed into continuous-infusion mode over 2 h for severe brain infections. Venous blood sample and CSF were also withdrawn for checking meropenem concentration at 4 h after dosing, and plasma fraction was separated immediately. The other (male) meningitis patient received an IV bolus of 2 g meropenem (Mepem®) for bacterial infection. Venous blood samples and CSF were withdrawn at 1 h, 2 h, 4 h and 8 h after dosing and plasma fraction was separated immediately.

3. Results and discussion

Biological samples usually consist of high concentrations of proteins, which greatly affect CE separation. In this study, C_{18} SPE cartridge was employed for sample pretreatment and cleanup of meropenem from human plasma. For CSF study, the sample by direct injection without sample pretreatment was used. Meropenem has two UV maximal absorptions at 200 nm and 300 nm. Plasma sample measurement could not be carried out at 200 nm because of interference from endogenous substances, although SPE cartridge was used for sample pretreatment. Therefore, the wavelength of 300 nm was chosen to study the analyte in plasma and in CSF. Optimization of the injection process for CSF samples and separation conditions were investigated.

3.1. CSF samples injection

Preliminary investigation of CSF sample injection, hydrodynamic, conventional electrokinetic and field-amplified sample stacking (FASS) methods was performed for the analysis of meropenem in CSF matrix, and results are shown in Fig. 2A–C, respectively. We found the FASS mode had the best sensitivity and symmetry peak shape of meropenem and cefotaxime, so it was chosen for use in the remainder of the CSF study. Firstly, the capillary was conditioned by the running buffer and then the water plug was introduced into the capillary by pressure. Secondly, cationic analytes were introduced into the capillary under positive voltage. Owing to the low conductivity of the water plug, the analytes slow down when they reach the boundary of the running buffer. The cations stack at the interface between the water plug and running buffer because of the amplified field. Finally, separation proceeds under positive voltage and the analytes migrate, allowing for detection by electrophoretic mobility. The capillary was dipped into water for 6 s and then a 34.5 mbar 6 s water plug from a different vial was inserted. Dipping the

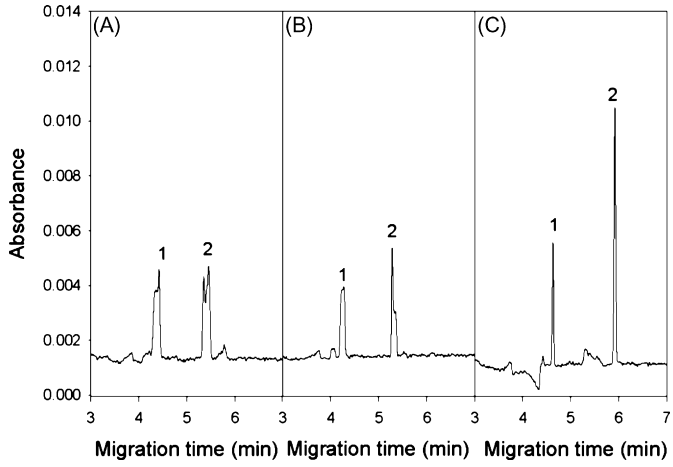


Fig. 2. Hydrodynamic injection (34.5 mbar, 25 s) (A); electrokinetic injection (5 kV, 65 s) without water plug (B); and electrokinetic injection (5 kV, 65 s) with water plug (34.5 mbar, 6 s) (C); of meropenem and cefotaxime in CSF. Peaks: 1, cefotaxime (IS); 2, meropenem. CE conditions: applied voltage, 15 kV (detector at cathode side); uncoated fused-silica capillary, 40.2 cm (effective length 30 cm) × 50 μm ID; wavelength, 300 nm.

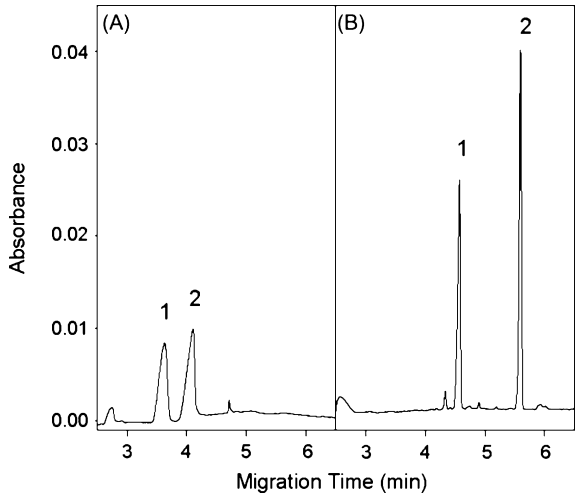


Fig. 3. Electropherogram for the analysis of meropenem and cefotaxime using different modes. (A) CZE, Tris 40 mM (pH 8.0); (B) MEKC, Tris 40 mM (pH 8.0) with SDS 200 mM. CE conditions: applied voltage, 15 kV (detector at cathode side); uncoated fused-silica capillary, 40.2 cm (effective length 30 cm) × 50 μm ID; sample size, 34.5 mbar, 10 s; wavelength, 300 nm.

capillary inlet end and electrode in a vial containing water was found to prevent contamination of the sample solution with the high conductivity-running buffer.

In principle, application of higher voltage and a longer injection time period should result in more solute injected resulting in more sensitivity. However, longer injection time over 90 s was found to yield a broader peak in our study. The CSF samples were injected using a voltage of 5 kV for 65 s. To examine the effect of the length of the water plug, different injection periods (0, 3, 6 and 10 s) by hydrodynamic injection of water (0.5 psi, 34.5 mbar) were investigated. There was no difference in resolution, but higher reproducibility and higher sensitivity were obtained when the water plug was used before the samples were injected. A water plug (34.5 mbar, 6 s) selected for separation provided the highest detection signal. With electrokinetic sample introduction, the amount of solute injected was proportional to the effective electrophoretic mobility. Thus, to charge the analytes, hydrochloric acid was added to the sample. It has already been demonstrated that the addition of 1 N hydrochloric acid to the sample matrix (v/v, 1:20) may enhance the protonation of the drug and the highest sensitivity detection was obtained.

3.2. Separation modes

Two modes of sample separation, capillary zone electrophoresis (CZE) and MEKC, were used to study the effect of the analyte in biological samples in the capillary. Separations of meropenem spiked in human plasma after SPE pretreatment were investigated. Fig. 3A and B present electropherograms obtained from CZE studied at 15 kV with 40 mM Tris (pH 8.0) and MEKC studied at 15 kV with 40 mM Tris (pH 8.0) and 200 mM SDS, respectively. Band broadening with low theoretical plate number of meropenem and cefotaxime was observed under CZE tested conditions (Fig. 3A). The anionic surfactant SDS added in Tris buffer yielded effective improvement of theoretical plate number. Comparing MEKC to CZE mode in terms of separation efficiency, an increase of approximately 20-fold and 13-fold theoretical plate number for meropenem and cefotaxime, respectively, was obtained at MEKC. Therefore, MEKC was selected for determination of meropenem in biological samples.

3.3. Optimization of separation conditions

The effects of the concentrations of Tris buffer (10–60 mM) were investigated in plasma spiked 50 μg/mL meropenem and 75 μg/mL cefotaxime (IS), using Tris buffer (pH 8.0) containing 200 mM SDS. The results can give similar resolution as shown in Fig. 4. There was an unclear change of peak shape of the meropenem at higher Tris concentrations, but a significantly broad peak width of meropenem was obtained at a concentration of Tris $\leq$20 mM. In a comparison of Tris concentrations (from 10 to 60 mM) on the effect of the sensitivity and theoretical plate for meropenem, the produced sensitivity ratios vary within a range from 0.53 to 1.0, and the best result was found to be 40 mM.

The degree of ionization of species present in the electrolyte system depends on the pH of the solution. Differences in the degree of ionization give rise to differences in electrophoretic and electroosmotic mobilities. Meropenem has one ionizable functional group (carboxylic acid) in structure; the dissociation of the carboxylic group with a pK_a value of 2.9. Therefore, for meropenem the anionic species dominates at the tested pH in electrolyte solution. The 40 mM Tris buffers with SDS (200 mM) at different pH levels (7.5, 8.0, 8.5, 9.0 and 9.5) for plasma were studied. Good resolution of the tested drug at various pH values was obtained. However, migration time decreased with increasing pH and a shorter migration time of the drugs was obtained at high pH. Closer migration between meropenem and cefotaxime (IS) was observed above 8.5. On the other hand, lesser response

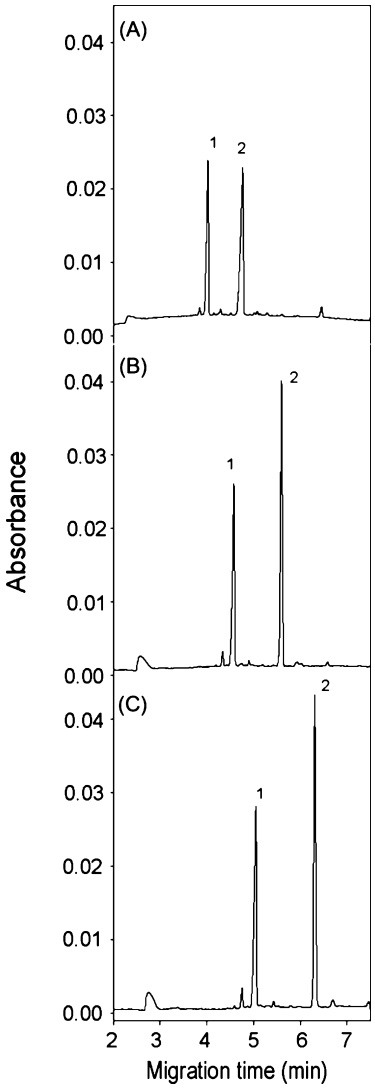


Fig. 4. Effect of concentration of Tris buffer (pH 8.0) with 200 mM SDS on the migration of meropenem and cefotaxime (IS) at 50 and 75 μg/mL, respectively, in human plasma. (A) 10 mM; (B) 40 mM; (C) 60 mM. Peaks: 1, cefotaxime (IS); 2, meropenem, respectively. For other CE conditions see Fig. 3.

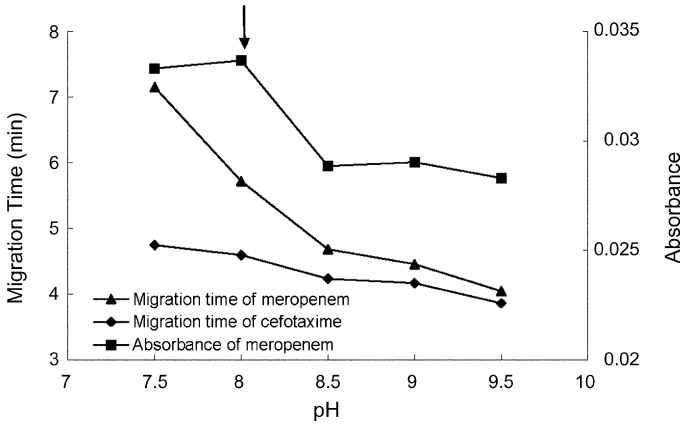


Fig. 5. Effect of pH values (7.5–9.5) in Tris buffer (40 mM) with SDS 200 mM on the migration of meropenem and the sensitivity.

of meropenem peak was obtained beyond pH 8.0. This may be due to partial decomposition of the drug under electrophoresis performed under more alkaline aqueous conditions (Fig. 5). Therefore, pH 8.0 of Tris buffer was chosen as optimal pH for determination of meropenem in plasma and in CSF.

In MEKC, the separation behavior is based on the homogeneous solution that can be a rapid establishment of partition equilibrium between the micelle and the aqueous phase. The effect of SDS at the concentration range of 50–250 mM in Tris buffer (40 mM; pH 8.0) on the separation was studied and the results indicated that electrophoresis of the drugs at 50 mM of SDS resulted in partial resolution of meropenem and cefotaxime (Fig. 6). The value of resolution (R_S) obtained for 50 mM SDS in Tris buffer was 0.95. With the concentration of SDS ≥100 mM, a baseline resolution of tested drug was observed. However, a significant peak shape improvement is obtainable by increasing the concentration of SDS, leading to sharper peaks and higher theoretical plate number. The SDS concentration at 200 mM was selected for this study. The optimization of the MEKC mode of CE conditions for analysis of meropenem was set at 40 mM Tris buffer (pH 8.0) with 200 mM SDS as a running buffer and the analyte was monitored at 300 nm. Under 15 kV as a separation voltage, the current (μA) produced about 70 μA in this background electrolyte. The typical electropherograms of the MEKC separation of meropenem and cefotaxime (IS) in plasma and in CSF are shown in Fig. 7A and D, respectively. Reproducibility of migration velocity of meropenem and cefotaxime in plasma was investigated, and observed migration times were 5.70 ± 0.16 min and 4.58 ± 0.10 min for meropenem and cefotaxime, respectively. The apparent mobility (μA) was calculated according to the equation: $\mu A = \mu E + \mu\mathrm{EOF} = (lL/tV)$ where l: length along the capillary (cm) to detector, V: voltage, t: migration time (s) and L: total length (cm) of the capillary [20]. Methanol was used for EOF determination. Under optimized CE conditions, the apparent mobility values of EOF, meropenem and cefotaxime (IS) were 4.44×10^{-4} cm^2/V s, 2.35×10^{-4} cm^2/V s and 2.93×10^{-4} cm^2/V s, respectively. The electrophoretic mobility values (μE) of meropenem and cefotaxime were -2.09×10^{-4} cm^2/V s and -1.51×10^{-4} cm^2/V s, respectively.

3.4. Validation of meropenem spiked in biological samples

To evaluate the quantitative applicability of the method, five different concentrations of meropenem over the range of 0.5–50 μg/mL with fixed concentration of 75 μg/mL or 30 μg/mL cefotaxime as IS in plasma and CSF, respectively, were studied. The linearity between the peak-area ratios (y) of the analyte to IS and the concentration of the analyte (x, μg/mL) was investigated. The linear regression equations in biological samples were obtained as follows: for meropenem in plasma assay ($n = 5$), $Y = (0.0288 \pm 0.0009)X - (0.0021 \pm 0.0012)$ for intra-day and $Y = (0.0289 \pm 0.0020)X - (0.0030 \pm 0.0074)$ for inter-day; for meropenem in CSF assay ($n = 5$), $Y = (0.0531 \pm 0.0008)X + (0.0322 \pm 0.0082)$ for intra-day and $Y = (0.0533 \pm 0.0012)X + (0.0401 \pm 0.0075)$ for inter-day. Good correlation coefficients of 0.999 were observed for all straight lines. The precision of the proposed method for spiked samples was stud-

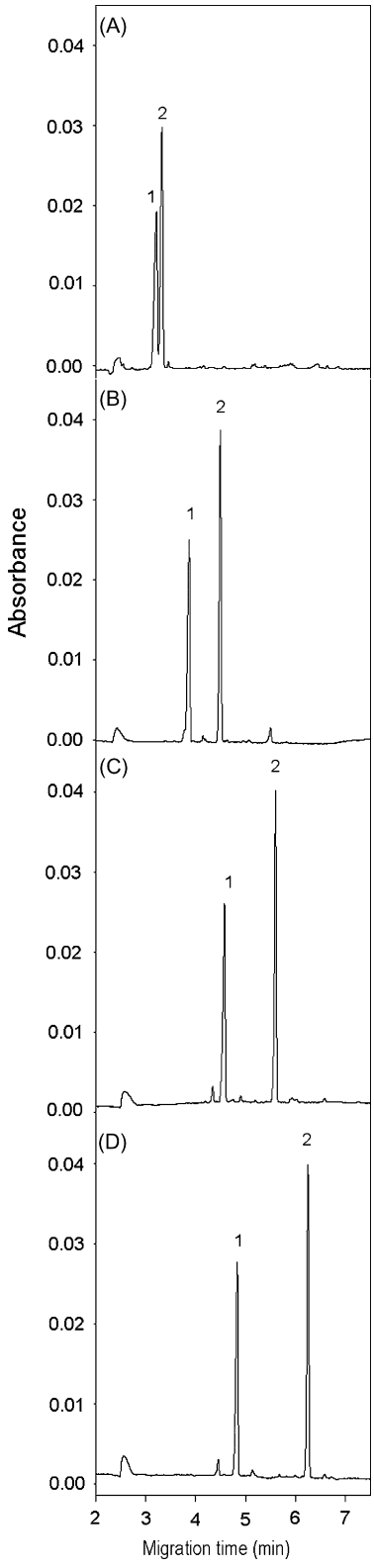


Fig. 6. Effect of SDS concentrations (50–250 mM) in Tris buffer (40 mM, pH 8.0) on the migration of meropenem and cefotaxime (IS) at 50 and 75 μg/mL, respectively, in human plasma. Electropherograms (A) 50 mM; (B) 150 mM; (C) 200 mM; (D) 250 mM. Peaks: 1, cefotaxime (IS); 2, meropenem, respectively. For other CE conditions see Fig. 3.

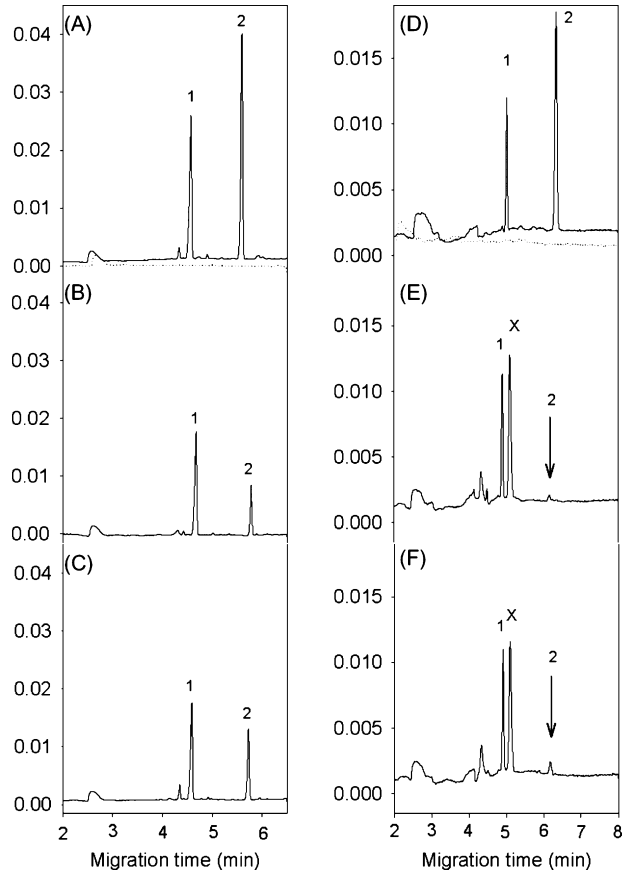


Fig. 7. Electropherograms of meropenem in plasma and CSF determinations. (A) plasma spiked 50 μg/mL; (B) IV bolus of 2 g meropenem after 4 h in plasma; (C) IV continuous-infusion of 2 g meropenem after 4 h in plasma; (D) CSF spiked 50 μg/mL; (E) IV bolus of 2 g meropenem after 4 h in CSF; (F) IV continuous-infusion of 2 g meropenem after 4 h in CSF. Peaks: 1, cefotaxime (IS); 2, meropenem; x: unknown peak, respectively. Dashed line is plasma blank or CSF blank. For other CE conditions see Fig. 3.

ied and evaluated as RSD and RE. The relative recoveries of meropenem were obtained from the calibration graph constructed from plasma and CSF spiked with different amounts of meropenem at low, medium and high concentration levels. The precision of the method for meropenem determination on intra- and inter-day analyses at three concentrations is shown in Table 1. RSD and RE were all less than 9.0%. Compared to the peak area ratio of standard meropenem, the absolute recoveries ($n = 4$) for meropenem at 50 μg/mL, 10 μg/mL and 1 μg/mL were about 67.8 ± 1.4, 67.9 ± 1.5 and $82.1 \pm 1.9\%$, respectively, and RSD at 50 μg/mL, 10 μg/mL and 1 μg/mL were 1.9, 2.3 and 2.3, respectively. The LOD of the proposed method for meropenem determination in plasma (S/N = 3, 34.5 mbar, 10 s) and in CSF (S/N = 3, 5 kV, 65 s) were found to be 0.2 μg/mL and 0.3 μg/mL, respectively.

3.5. Application

One of the most important mainstays in the therapy of severe brain infections is the administration of antimicrobial agents that are known to reach effective concentrations in the CSF. This issue becomes a particularly important link to the development

Table 1
Precision and accuracy for the recovery of spiked meropenem in plasma and in CSF

Concentration known (μg/mL)	Concentration found (μg/mL)	RSD (%)	RE (%)
Plasma			
Intra-day[a] ($n=5$)			
1.0	0.98 ± 0.03	3.1	−2.0
10.0	9.82 ± 0.04	0.4	−1.8
50.0	49.88 ± 0.07	0.1	−0.2
Inter-day[a] ($n=5$)			
1.0	1.02 ± 0.05	4.9	2.0
10.0	10.06 ± 0.39	3.8	0.6
50.0	49.93 ± 1.11	2.2	−0.1
CSF			
Intra-day[a] ($n=5$)			
1.0	0.95 ± 0.07	7.4	−5.0
15.0	15.37 ± 0.18	1.2	2.5
50.0	50.47 ± 2.23	4.4	0.9
Inter-day[a] ($n=5$)			
1.0	0.91 ± 0.08	8.8	−9.0
15.0	14.81 ± 0.57	3.8	−1.3
50.0	49.47 ± 1.00	2.0	−1.1

[a] Intra-day data were based on five replicate analyses and inter-day were from five consecutive days.

of bacterial resistance to subinhibitory antibiotic concentrations at the target site.

A female patient with bacterial meningitis received IV bolus of 2 g meropenem over 20 min every 8 h in ICU by traditional dosing interval treatment. Several days later, meningeal inflammation of the female patient had not subsided. Therefore, 2 g every 8 h dosing interval for patient by IV bolus shifted to continuous-infusion mode over 2 h for severe infection condition. The concentrations of meropenem by IV bolus (over 20 min) and by continuous-infusion (over 2 h) in adult with meningitis were determined in plasma and in CSF by the MEKC method. The electropherograms of plasma samples by IV bolus or continuous-infusion of the drug after 4 h are shown in Fig. 7B and C, respectively, and concentrations of meropenem were 13.95 μg/mL and 22.61 μg/mL, respectively. The electropherograms of CSF by IV bolus or continuous-infusion of the drug after 4 h dosing are shown in Fig. 7E and F, respectively, and concentrations of meropenem in CSF were 1.25 μg/mL and 2.64 μg/mL, respectively. The concentration of meropenem after 4 h dosing for plasma as well as for CSF were different after IV bolus and IV continuous-infusion. Moreover, administration of meropenem in continuous-infusion (dripping) in ill patients with meningitis appeared to provide higher concentrations of the drug. Exceeding the MIC over a dosing interval ($t > \text{MIC}$) was considered for favorable clinical and bacteriological outcome. Continuous-infusion of the antibiotics could reach this value to exert a significant antimicrobial effect. The other male meningitis patient received meropenem vial 2 g (Mepem®, 0.5 g/vial, Sumitomo) from IV bolus (over 20 min) for severe infection. We measured the plasma and CSF samples from the meningitis patient at 1 h, 2 h, 4 h and 8 h after administration. The concentration–time curves of meropenem in plasma and CSF after IV bolus are shown in Fig. 8. The concentrations of meropenem in plasma were 45.04, 23.48, 5.64 and 1.20 μg/mL at 1 h, 2 h, 4 h, and 8 h, respectively. The concentrations of meropenem in CSF were 1.75, 2.61, 2.64 and 1.88 μg/mL at 1 h, 2 h, 4 h and 8 h, respectively. From the results, elimination of meropenem from the CSF space was slower than elimination from plasma.

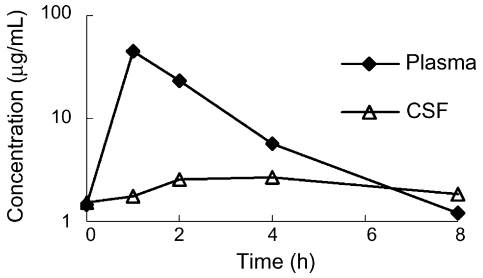


Fig. 8. Concentration–time curves of meropenem in plasma and in CSF after IV bolus of 2 g meropenem.

The meropenem was stable in biological matrix for at least 30 days when frozen at −40 °C. The MEKC method was suitable for the analysis of meropenem in plasma and in CSF collected during pharmacokinetic and pharmacodynamic investigations in humans. Meropenem is commonly used in serious bacterial infections. The serum half-life is prolonged in the elderly and in those with renal function insufficiencies. An individualized efficacy therapy and minimized toxicity for patient safety, dose-adjustment of meropenem in the elderly or in renal failure patients can obtain better antibacterial efficacy, based on individual pharmacokinetic parameters. The characteristics of the small amount sample volume needed and high sensitivity of this MEKC analytical method for quantitation of meropenem in biological samples make it very useful in clinical practice.

4. Concluding remarks

The SPE pretreatment for plasma and direct injection for CSF coupled to MEKC for determination of meropenem in biological samples described here represented a sensitive and efficient analytical method. To separate the test drug from endogenous components, we used the CE method based on anionic surfactant SDS as a micelle and detection was achieved at 300 nm. Validation of the method for quantitation of meropenem in plasma and in CSF showed the method has high sensitivity and accuracy for clinic use. Therefore, the proposed MEKC method was suitable for analysis of meropenem in plasma and CSF collected to assess whether sufficient drug concentrations in CSF can be counted upon.

Acknowledgements

The authors are grateful to the National Science Council, ROC, for financial support of the study (NSC 95-2113-M-037-001). The authors also acknowledge General Clinical Research Center of Kaohsiung Medical University Hospital for CE experiments.

References

[1] W.A. Krueger, T.H. Schroeder, M. Hutchison, E. Hoffmann, H.J. Dieterich, A. Heininger, C. Erley, A. Wehrle, K. Unertl, Antimicrob. Agents Chemother. 42 (1998) 2421.

[2] S.R. Norrby, P.A. Newell, K.L. Faulkner, W. Lesky, J. Antimicrob. Chemother. 36 (1995) 185.

[3] T. Kutluk, O. Kurne, C. Akyüz, M. Ceyhan, G. Kanra, M. Büyükpamukcu, G. Secmeer, Pediatr. Blood Cancer. 42 (2004) 284.

[4] J.G. Hardman, L.E. Limbird, A.G. Gilman (Eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th ed., McGraw-Hill Medical Publishing Division, New York, 2001, p. 1214.

[5] J.R. Edwards, P.J. Turner, Scand. J. Infect. Dis. 96 (1995) 5.

[6] D.G. Waller, A.G. Renwick, K. Hillier, Medical Pharmacology and Therapeutics, second ed., Elsevier, New York, 2005, 584.

[7] D. Farin, R. Kitzes-Cohen, G. Piva, I. Gozlan, Chromatographia 49 (1999) 253.

[8] B.A. Christensson, I. Nilsson-Ehle, M. Hutchison, S.J. Haworth, B. Öqvist, S.R. Norrby, Antimicrob. Agents Chemother. 36 (1992) 1532.

[9] M. Ehrlich, F.D. Daschner, K. Kümmerer, J. Chromatogr. B. 751 (2001) 357.

[10] H. Elkhaïli, S. Niedergang, D. Pompei, L. Linger, D. Leveque, F. Jehl, J. Chromatogr. B. 686 (1996) 19.

[11] Y. Özkan, İ. Kűcűkgűzel, S.A. Özkan, H.Y. Aboul-Enein, Biomed. Chromatogr. 15 (2001) 263.

[12] J.L. Kuti, C.H. Nightingale, R.F. Knauft, D.P. Nicolau, Clin. Ther. 26 (2004) 493.

[13] C. Robatel, L.A. Decosterd, J. Biollaz, P. Eckert, M.D. Schaller, T. Buclin, J. Clin. Pharmacol. 43 (2003) 1329.

[14] C. Robatel, T. Buclin, P. Eckert, M.D. Schaller, J. Biollaz, L.A. Decosterd, J. Pharm. Biomed. Anal. 29 (2002) 17.

[15] Y.L. Chang, M.H. Chou, M.F. Lin, C.F. Chen, T.H. Tsai, J. Chromatogr. A. 961 (2002) 119.

[16] R. Nau, C. Lassek, M. Kinzig-Schippers, A. Thiel, H.W. Prange, F. Sörgel, Antimicrob. Agents Chemother. 42 (1998) 2012.

[17] S. Taniguchi, K. Hamase, A. Kinoshita, K. Zaitsu, J. Chromatogr. B. 727 (1999) 219.

[18] Y. Mrestani, R. Neubert, F. Nagel, J. Pharm. Biomed. Anal. 20 (1999) 899.

[19] T. Kitahashi, I. Furuta, J. Chromatogr. Sci. 43 (2005) 430.

[20] K.D. Altria (Ed.), Capillary Electrophoresis Guidebook: Principle, Operation, and Application, Humana Press Inc., New Jersey, 1996, p. 7.