Determination of Meropenem by Capillary Electrophoresis Using Direct Injection of Serum

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Abstract

Concentration determination of meropenem, a carbapenem antibiotic, using a capillary electrophoresis method by direct injection of serum samples without any pretreatment is described herein. Sodium tetraborate (25mM)–sodium hydroxide (0.1M) containing sodium dodecyl sulfate (90mM) is used as a run buffer (pH 10.0). Meropenem is detected at its absorption maximum at 297 nm. Migration time of meropenem is approximately 7.2 min, and the detection limit of the assay is 2.0 mg/L at a signal-to-noise ratio of 3.0. The relative standard deviations of intra- and interassay accuracies are 3.43–8.87% and 4.28–8.54%, respectively, at a nominal concentration of 6.3–100.0 mg/L, and their recovery rates are 94–111% and 92–105%, respectively.

Introduction

Meropenem is a carbapenem antibiotic (Figure 1). Traditional carbapenem antibiotics required that a human renal dehydropeptidase I (DHP-I) inhibitor for maintaining antimicrobial activity or agent for reducing renal toxicity should be included. For meropenem, stability against DHP-I is improved by the introduction of a methyl group into the 1β position of the carbapenem skeleton. Additionally, this drug is designed to have strong antimicrobial activity and to maintain this antimicrobial property against gram-negative microbes (including *Pseudomonas aeruginosa*) by the introduction of a dimethylcarbamoyl pyrrolidinyl group into the second position, through the sulfur atom. At the same time, it is designed to have reduced renal toxicity as well as a reduced spasm-inducing effect. Furthermore, this antibiotic also has an antimicrobial spectrum against gram-positive microbes, anaerobes and *Staphylococcus aureus* and good stability against β-lactamases of various bacteria (1). Its side effects are likely to arise in cases in which creatinine clearance is low (equal to or less than 30 mL/min) because of renal dysfunction, or for aged people with reduced physiological functions. Also, drug monitoring is essential for controlling its concentration in blood.

Meropenem undergoes metabolism, giving a microbiologically inactive β-lactam ring-opened product that is mainly eliminated as an unchanged drug by the kidneys (2). Therefore, a simple and easy determination method of meropenem is required. Traditionally, several methods have been reported using high-performance liquid chromatography (HPLC) (3–9). As for pretreatment methods of serum samples, deproteinization with an acid solvent (3), solid-phase extraction using a cartridge column (4), dilution (5), and online column switching (6,7) have been reported. Of these, the online column switching method enables fast determination by direct injection of serum samples, but it makes the measuring system and its maintenance and control complicated. Besides, it poses problems such as the extraction column being lower than that of the capillary tube in durability.

Determination of meropenem in plasma, urine, and injection using capillary electrophoresis (CE) has been reported (10,11). Mrestani et al. (10) performed an analysis using a 10mM phosphate buffer (pH 7.2) as a run buffer. As for sample preparation, they deproteinized plasma samples with acetonitrile and used urine samples without pretreatment. Acetonitrile has been widely used for deproteinization for CE (12) and, in performing sample preparation, appropriate internal standards need to be selected to compensate accuracy and precision of determination. Determination by a serum (or plasma) direct injection method should be performed without interference from endogenous substances, including proteins, which account for the majority of substances in serum (13,14).

Micellar electrokinetic capillary chromatography (MECC) is suitable for analyzing drugs in body fluids (15,16). Therefore, the approach of this study was to develop a new meropenem determination method that is simple, easy, fast, and highly precise using MECC by direct serum injection. Described is a meropenem analysis method using MECC by direct serum injection, as well as its validation of the assay.

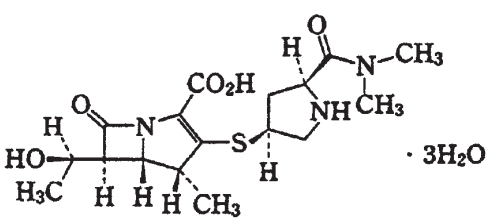

Figure 1. The chemical structure of meropenem.

Experimental

Reagents and chemicals

Meropenem was obtained from Sumitomo Pharmaceuticals (Osaka, Japan). Sodium tetraborate, sodium dodecyl sulfate (SDS), and sodium hydroxide (0.1M) were purchased from Wako Pure Chemicals (Osaka, Japan). All solvents and chemicals used were at least of analytical grade.

Buffer preparation

A 25mM sodium tetraborate solution was prepared, and sodium hydroxide (0.1M) was added so that it was adjusted to pH 10.0. SDS was then added so that its concentration in the buffer became 90mM. This run buffer was filtered through a 0.45-µm membrane (Millipore, Bedford, MA) and deaired ultrasonically for 5 min before actual use.

Apparatus and analysis conditions

MECC was performed with a P/ACE system MDQ (Beckman Coulter, Fullerton, CA). An untreated fused-silica capillary tube made by the same company of 500-mm effective length and 75-µm bore was used and stored at 20°C. The injection time was 5 s (0.5 Pa) at the anode, load voltage was 25 kV, and detection was carried out at 297 nm (at the cathode). After each analysis of one sample, the capillary was rinsed with sodium hydroxide (0.1M) for 3 min and then with run buffer for 5 min (both under 20 Pa pressure), and then another serum sample was directly injected.

Quantitation

The stock solution (1.0 g/L) was prepared by dissolving 57.04 mg of meropenem reference standard in 50 mL of purified water. This stock solution was stable for over 3 months even when stored at 4°C. Working solutions were prepared daily by dilution of the stock solution. The serum solution was prepared by adding meropenem standard solution to drug-free human serum so that the concentration of the meropenem standard solution became 5% (v/v). Serum standard solutions of 0, 3.1, 6.3, 12.5, 25.0, 50.0, 100.0, and 200.0 mg/L were prepared as calibration standards. Linearity was evaluated by plotting the peak area against each concentration in order to obtain the linear regression. The limit of detection was defined as the meropenem concentration yielding a peak three times the noise level. The influence of endogenous substances in serum on the meropenem determination was evaluated by comparing electropherograms of drug-free human serum and the serum standard solution. To obtain intra- and interassay accuracy, meropenem serum standard solutions of various concentrations were prepared. Each solution was then measured five times in a serial manner for intra-assay accuracy, and a sample of each concentration was measured twice a day for 6 days in a repetitive manner for interassay accuracy, and average values were used. Finally, relative standard deviations (RSDs) of intra- and interassay accuracies were calculated for each concentration. Samples were stored at 4°C during this procedure. Recovery was determined by comparing the peak area resulting from serum standard solution to the peak area resulting from an aqueous solution at the same meropenem concentration injected directly into the capillary tube.

Results and Discussion

According to an evaluation of the results of optimal pH value of the run buffer at pH 8.0–11.0 (the standard condition was 100mM borate buffer containing 50mM SDS), sensitivity (peak area) became the highest at pH 8.0 and, additionally, migration time became shorter, but peak shape became broad and tailing occurred. Therefore, quantitation could not be performed. The

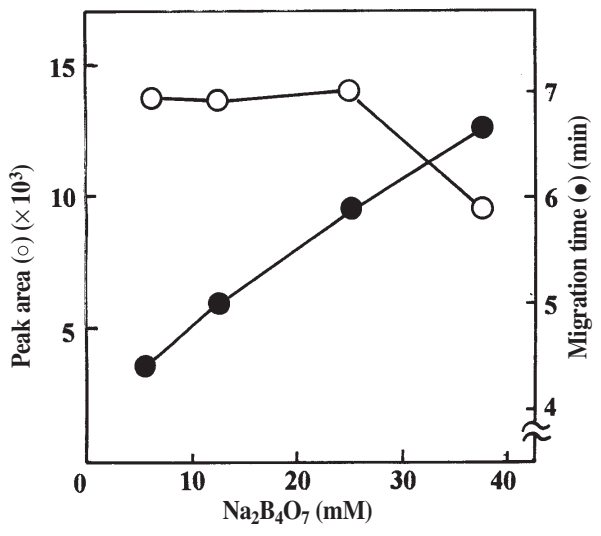

Figure 2. The effect of sodium tetraborate concentration on peak area and the migration times of meropenem. Run buffer, sodium tetraborate (pH 10.0) containing SDS (50mM); applied voltage, 25 kV; capillary temperature, 25°C; pressure injection time, 5 s; UV detection wavelength, 297 nm; and concentration of standard serum, 50.0 mg/L.

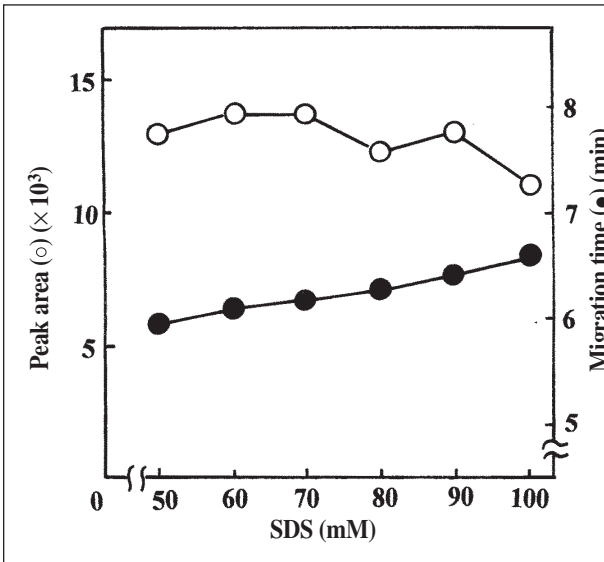

Figure 3. The effect of SDS concentration in run buffer on peak area and the migration times of meropenem. Run buffer, sodium tetraborate (25mM, pH 10.0) containing SDS; applied voltage, 25 kV; capillary temperature, 25°C; pressure injection time, 5 s; UV detection wavelength, 297 nm; and concentration of standard serum, 50.0 mg/L.

evaluation revealed pH 10.0 to be optimal. Evaluation results of sodium tetraborate concentration are shown in Figure 2.

The higher the ionic strength that the buffer reached, the more band diffusion of peak could be suppressed, resulting in better resolution as well as sharper peaks and leading to a higher plate number (17). However, as the ionic strength of the buffer increased, the electric current loaded on the capillary increased too, and measurement could not be performed at equal or greater than 40mM. Therefore, 25mM sodium teraborate was optimal for this method. Evaluation results of the concentration of SDS to be used for MECC are shown in Figure 3.

In order for meropenem to be separated from other serum substances so that its peak shape became sharp, the SDS concentration needed to be set high. Good separation was achieved at an SDS concentration of 90mM. Sharp changes in sensitivity and migration time were observed depending on the capillary temperature (Figure 4). The higher the capillary temperature became, the higher the baseline level of electropherogram became and, accordingly, the electric current became higher and sensitivity decreased.

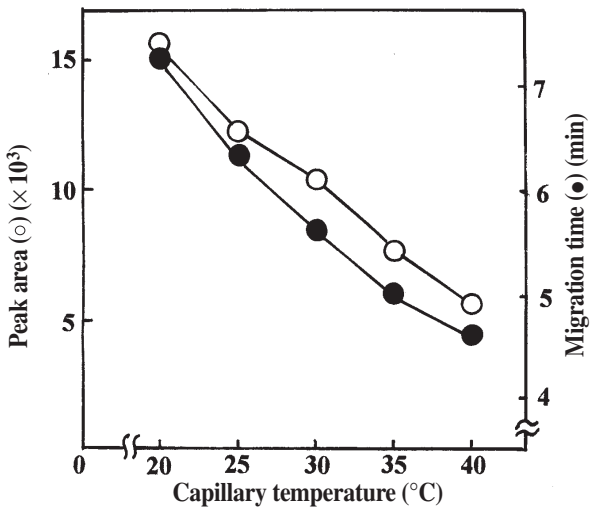

Figure 4. The effect of capillary temperature on peak area and the migration times of meropenem. Run buffer, sodium tetraborate (25mM, pH 10.0) containing SDS (90mM); applied voltage, 25 kV; pressure injection time, 5 s; UV detection wavelength, 297 nm; and concentration of standard serum, 50.0 mg/L.

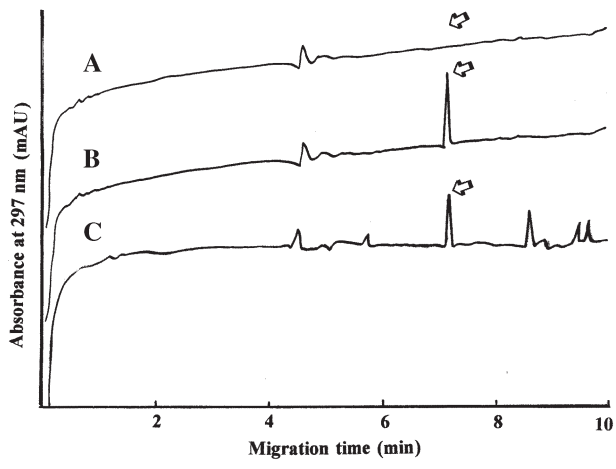

Figure 5. Typical electropherograms of meropenem in blank human serum (A), human serum spiked with 25.0 mg/L meropenem (B), and a serum sample from a patient on meropenem (15.9 mg/L) (C). Run buffer, sodium tetraborate (25mM, pH 10.0) containing SDS (90mM); applied voltage, 25 kV; capillary temperature, 20°C; pressure injection time, 5 s; and UV detection wavelength, 297 nm.

The capillary temperature was set at 20°C so that highly sensitive and rapid measurement could be performed. Injection time has a big influence on measurement sensitivity. Generally, the longer the injection time becomes, the more sensitivity increases. Evaluation results of injection time varying 5–10 s for this method showed that when injection time was equal to or over 6 s, almost no change was observed in peak height of meropenem, peak width became broad, and peak shape became imperfect with the peak top split in two. Thus, the injection time of 5 s was believed to be the limit because serum was directly injected with this method.

As for measurement wavelength, there have been reports in which determination of meropenem concentration in serum was performed at 296–308 nm (3,5–7) and 208 nm (4) by HPLC and 303 nm (10) by CE. Mrestani et al. (10) reports that detection can be carried out even at 200 nm if a serum sample is deproteinized with acetonitrile. According to the evaluation with this method, meropenem exhibited the maximum UV region absorption (λ max) at equal or under 200 and 297 nm. The maximum sensitivity was obtained at 200 nm through measurement of the aqueous standard solution of meropenem, although with a serum sample measurement could not be carried out because of interference from endogenous substances in serum in its wavelength. Therefore, measurement was carried out at 297 nm with this method.

There is nothing about the structure of meropenem that makes it amenable for quantitation by MECC without the need for an internal standard. Generally, no internal standard was necessary for the serum direct injection method because serum pretreatment operation causing the measurement errors was not performed.

Table I. Statistics of Spiked Serum Samples for Meropenem

Nominal concentration (mg/L)	Measured concentration (mg/L)	RSD (%)	Recovery (%)
Intra-assay (*n* = 5)			
6.3	6.2	8.87	98.4
12.5	13.9	6.77	111
25.0	25.7	7.35	103
50.0	47.2	7.84	94.4
100.0	93.6	3.43	93.6
Interassay (6 days)			
6.3	5.8	5.17	92.1
12.5	12.6	6.54	101
25.0	26.0	8.54	104
50.0	52.4	5.04	105
100.0	99.7	4.28	99.7

Inversely, accuracy might decrease by the interaction of an internal standard and serum proteins. Therefore, an internal standard was not used in this method. Figure 5 shows electropherograms of blank serum, meropenem standard serum, and a case in which a patient was administered with intravenous drip infusion containing 0.5 g of Meropen (meropenem, Sumitomo Pharmaceuticals, Osaka, Japan).

Analysis could be performed without interference from endogenous substances in serum. Linearity of 0–200 mg/L was good (r^2 = 0.999). The detection limit of the assay was 2.0 mg/L at a signal-to-noise ratio of 3. It has been reported to be 0.025–2.5 mg/L by HPLC (3–7) and 0.5 mg/L by CE (10).

The measurement wavelength, pretreatment, and determination methods were different according to reported literatures. Therefore, measurement methods were compared, respectively. Robert et al. (4) prepared the concentration-time curve of meropenem regarding a patient administered with 1000 mg (three times per day) of meropenem and reported that its concentration in venous plasma was 10–55 mg/L and that the minimal inhibitory concentration of susceptible germs was 4 mg/L.

The detection limit of the assay of this method is believed to be applicable to drug monitoring. Table I shows the evaluation results of intra- and interassay precisions and absolute recovery. Using HPLC, the RSD of intra-assay has been reported to be 3.5–5.9% (14.7–63.3 mg/L) (4), 2.14–9.80% (1.1–97.1 mg/L) (6), and 2.2–6.0% (0.9–29.6 mg/L) (7). That of the interassay has been reported to be 2.3–3.6% (14.1–62.2 mg/L) (4). Recovery has been reported to be 87.2–93.4% (concentration for addition: 15.0–60.0 mg/L) (4), 98.5% (6), and 89.4–105.2% (0.9–29.6 mg/L) (7). It can hardly be said that accuracy and precision of measurement of this method is inferior compared with those of the reported results.

Conclusion

This method is characterized by the fact that measurement is possible by direct injection of serum samples into the capillary. This has enabled fast, reliable, and economical analysis. Because it requires serum and buffer of microquantity, reduction in blood volume to be collected and substantial decrease in liquid waste disposal arising from analysis were achieved. Measurement with a CE system is easy in operation because it is performed fully automatically and moreover has no scope for inconsistency in measurements and measurement errors among the measurers because it does not require pretreatment of serum samples.

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