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Capillary zone electrophoresis determination of meropenem in biological media using a high sensitivity cell

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Abstract

A capillary electrophoresis method with a high sensitivity cell (Z-cell) has been developed for the determination of meropenem in aqueous solution and in biological media (urine, plasma). Water samples were analysed using two calibration curves of meropenem with standard capillary and a capillary with a high sensitivity cell. In urine, the samples were only diluted in buffer and were injected without any further sample preparation. For the analysis of plasma samples, a calibration curve was utilized covering the meropenem concentration range of 0.5–200 μg/ml. The detection limit and the relative standard deviation of the migration times and of the peak areas were determined.
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Keywords: Capillary electrophoresis with high sensitivity cell; Meropenem

1. Introduction

The determination of antibiotics in biological fluids is often performed by microbiological procedures [1,2]. In recent years, liquid chromatography (LC) has proved to be a powerful analytical tool for measuring such drugs in biological fluids, because of its specificity, rapidity and sensitivity [3,4]. Until now, cephalosporin antibiotics were measured using micellar electrokinetic capillary chromatography (MECC) and capillary zone electrophoresis (CZE) [5–8]. Meropenem as a new antibiotic agent was determined by high-performance liquid chromatography [9,10] as well as by microbiological methods [11]. Capillary zone electrophoresis (CZE) with a high sensitivity cell turned out to be a new technique for meropenem analysis. The samples were only diluted with buffer and then injected without any further sample preparation (urine sample). The performance of the CZE approach for the determination of meropenem was tested by measuring the detection limit at 200 and 303 nm (direct injection of the plasma sample) and the relative standard deviation of the migration times and of the peak areas.

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The method developed was used to measure meropenem in plasma of patients.

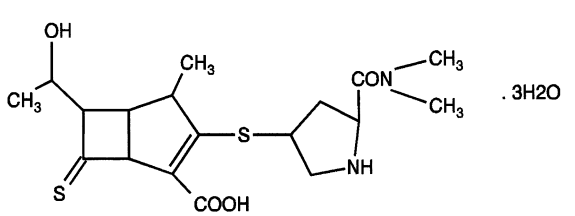
2. Experimental

2.1. Apparatus

Capillary electrophoresis experiments were performed on a Hewlett Packard Model G1600A (Waldbronn, Germany) 3D CE system with diode-array detector from 190 to 600 nm. A CE ChemStation equipped with a HP Vectra 486/66U workstation was used for instrument control, data acquisition, and data analysis. The system was controlled by Windows software, which was modified to the HP system. The detection wavelengths were 200 and 303 nm. A standard capillary (fused-silica) with a total length (80.5 cm), length to detector (72 cm) and internal diameter (75 μm) and a capillary with high sensitivity cell (Z-cell) obtained from Hewlett Packard (Waldbronn, Germany) with a 8.5-cm outlet, 72-cm effective length and 75-μm i.d. and with a pathlength of the detection cell of 1 mm were used for the determination of meropenem.

2.2. Chemicals

Meropenem (Fig. 1) was obtained from Grünenthal (Germany). Acetone, methanol, ethanol, acetonitrile, potassium hydrogenphosphate and potassium dihydrogenphosphate were obtained from Merck (Darmstadt, Germany).



Meropenem

Fig. 1. Chemical structure of meropenem.

2.3. Sample preparation

Standard solutions of meropenem were prepared from 0.1 to 200 μg/ml in 10 mM buffer solution at pH 7.2. Human urine was diluted with standard solutions containing a known amount of meropenem (1:3, v:v). The urine samples were filtered through a 0.45-μm syringe filter and injected immediately into the apparatus. Plasma samples (1 ml) containing 0.5–200 μg/ml meropenem were mixed with 3 ml acetonitrile. These mixtures were centrifuged at $2200 \times g$ for 10 min. The samples were filtered through a 0.45-μm syringe filter and injected.

2.4. Buffer preparation

For capillary electrophoresis, a 10 mM phosphate buffer solution (pH 7.2) was prepared by dissolving 1.05 g potassium hydrogenphosphate and 0.53 g potassium dihydrogenphosphate in water, and making up to a volume of 1000 ml. The pH of the buffer was measured at 25°C using a HI 9321 microprocessor pH meter (HANA Instruments). The buffer solution was filtered through a 0.45-μm syringe filter and degassed by ultrasound for at least 10 min before use.

2.5. Recovery study

Four plasma samples were spiked with 5 and 50 μg/ml of meropenem. They were assayed and the resulting peak areas were compared with peaks resulting from aqueous solutions at the same concentrations.

2.6. Analysis conditions

A new capillary was washed for 10 min with NaOH (1.0 M) at 40°C, followed by washing for 10 min with water at the same temperature and for 5 min with water at 25°C. Before each injection, the capillary was flushed with 0.1 M NaOH for 5 min and with the actual buffer solution for 5 min. The temperature was kept at 25°C, and a separation potential of 30 kV was used. Acetone was used as a marker substance for the determination of the electroosmotic mobility. The sam-

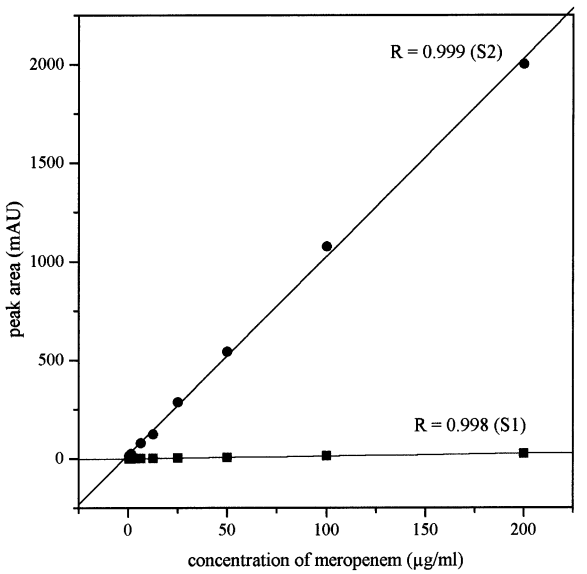


Fig. 2. Calibration graph for determination of meropenem with standard capillary (S1) and capillary with high sensitivity cell (S2).

ples (buffer/acetone, 99:1) were injected at a pressure of 50 mbar for 5 s (hydrodynamic injection). Detailed experimental conditions are given in the legends to the figures.

3. Results and discussion

In this paper we studied the separation and determination of meropenem in aqueous solution and in biological media. The determination of meropenem in aqueous solution was performed using standard capillary as well as capillary with a high sensitivity cell. The results showed that the application of a capillary with high sensitivity cell led to an improvement of the detection limit of about 10-fold over standard capillaries (Fig. 2).

CZE utilizing high sensitivity cell is very suitable for analysis of meropenem due to its UV absorption and good solubility in water. This is also the case for analysis of meropenem present in biological tissues and other body liquids at low concentrations. In our studies, meropenem had a negative electrophoretic mobility and migrated in the direction of the cathode (Table 1). The effective mobilities and the electroosmotic mobility were calculated at pH 7.2 as described by Weinberger [12]. The determination of meropenem in urine was performed at a pH of 7.2. The results of these measurements showed that at this pH, meropenem was separated from urine components and detected (Fig. 3). For measuring meropenem in urine, the samples for CZE were only diluted in buffer 1:3 and were injected into the apparatus without any further sample preparation. Freshly prepared samples were used and the capillary was flushed first with 0.1 M NaOH for 3 min and then with the actual buffer solution for 5 min.

In plasma, meropenem could also be analysed quantitatively at two wavelengths as described below. (1) Meropenem had two UV absorption maxima, at 200 and 303 nm whereas the plasma components exhibited UV absorption only at 200 nm in that range where meropenem was detected indicating that meropenem could be determined at 303 nm. In this case, we can determine meropenem in plasma at 303 nm through direct injection without any further sample preparation. For measuring meropenem in plasma, the samples were only diluted in water 1:4 and were injected into the apparatus. The detection limit in plasma through direct measurement at 303 nm is 4 µg/ml

Table 1
Results of analyses of meropenem with CZE[a]

	μ_e	R.S.D. (f) (%)	R.S.D. (t) (%)	Detection limit (µg/ml)
Water	-7.32 ± 0.022	0.3	0.5	0.1
Plasma	-7.86 ± 0.064	0.1	3.0	0.5
Urine	-7.42 ± 0.011	0.1	2.0	0.3

[a] μ_e, effective mobility (10^{-5} cm^2 V^{-1} per s); R.S.D. (f), relative standard deviation of the peak areas; R.S.D. (t), relative standard deviation of the migration times.

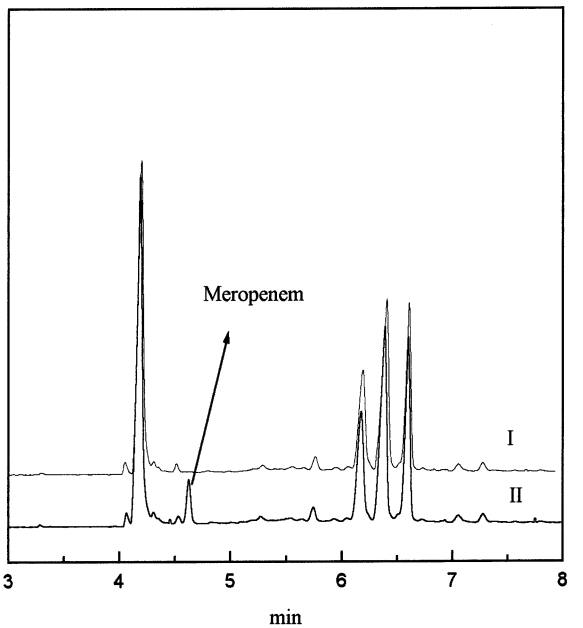


Fig. 3. Electropherogram of a blank human urine standard (I) and a urine sample containing 10 μg ml^{-1} of meropenem (II). Buffer: pH 7.2, 10 mM phosphate; capillaries with high sensitivity cell with 8.5-cm outlet, 72-cm effective length and 75-μm i.d.; 30 kV; temperature, 25°C; pressure injection, 5 s at 50 mbar; detection, 200 nm.

and the relative standard deviation of the migration times is 5% and of the peak areas 4% at a concentration of 10 μg/ml. The direct determination of meropenem in plasma using CZE has some advantages compared to LC, particularly concerning sample preparation. Usually, for the determination of meropenem in plasma by LC, it is essential to precipitate the protein component before the measurement. (2) At 200 nm, it is necessary to precipitate the plasma components. The treatment of the plasma samples with methanol and ethanol (1:3, v:v) led to a lower separation of meropenem from plasma components (Fig. 4).

Good results were achieved through the treatment of the plasma samples with acetonitrile (1:3, v:v) (Fig. 5). Fig. 5 shows electropherograms of a blank plasma standard and of a 10 μg/ml meropenem standard at pH 7.2. The electropherograms demonstrate that the meropenem peak is completely separated from plasma components.

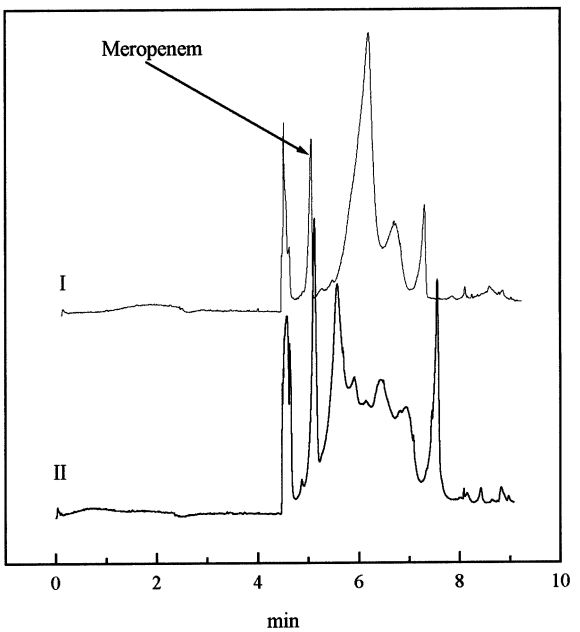


Fig. 4. Electropherogram of plasma treament with methanol (I) and with ethanol (II). For conditions see Fig. 3.

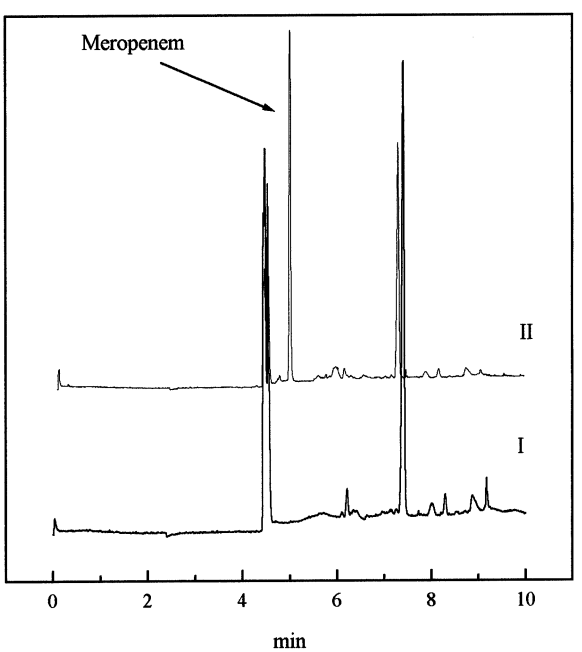


Fig. 5. Electropherogram of a blank plasma standard (I) and a plasma sample containing 10 μg ml^{-1} of meropenem (II). For conditions see Fig. 3.

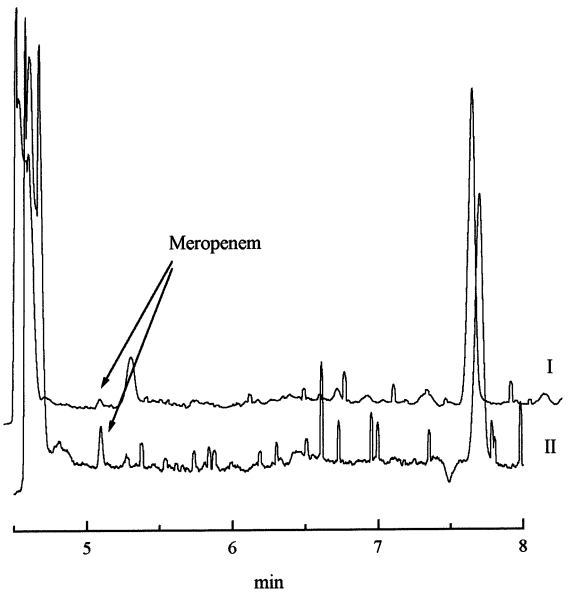


Fig. 6. Determination of meropenem in plasma of female patient 5-h-old, after i.v. administration. I: Concentration of meropenem (4.8 μg/ml) after 4 h administration. II: Concentration of meropenem (13.95 μg/ml) after 8 h administration. For conditions see Fig. 3.

The calibration curve of the area was linear ($y = 10.1x + 20.1$) with a correlation coefficient of $R = 0.999$. Mean recoveries from spiked samples were 98.6% ($n = 4$) at a meropenem concentration of 5 μg/ml and 97.8% ($n = 4$) at a concentration of 50 μg/ml by comparison with meropenem standard solutions of equivalent concentrations. To control the reproducibility of the peak areas and of the migration times, five injections of meropenem (10 μg/ml) were made for these samples. Using this method, good reproducibility of the peak areas and of the migration times (Table 1) was obtained.

Furthermore, the concentration of meropenem in plasma of three patients was determined after i.v. administration of meropenem. Fig. 6 shows an electropherogram of plasma for patients 8 h after i.v. administration.

4. Conclusions

Capillary zone electrophoresis has been developed as a new method for the determination of meropenem in biological media. The CZE technique utilizing the Z-cell is easy to handle and shows good reproducibility. The high sensitivity, the low amount of sample required and the relatively short analysis time were the main advantages of the method. Therefore, this technique will be useful for clinical and medical researchers who are interested in measuring meropenem in urine and plasma samples.

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