Communications to the Editor

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SEPARATION AND DETERMINATION OF CEFPIRAMIDE IN HUMAN PLASMA BY ELECTROKINETIC CHROMATOGRAPHY WITH A MICELLAR SOLUTION AND AN OPEN TUBULAR-FUSED SILICA CAPILLARY

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Electrokinetic chromatography was used to determine cefpiramide (CPM) in human plasma, using antipyrine (AP) as an internal standard. A plasma sample was introduced into a fused silica capillary. Then high voltage was applied between the ends of the capillary dipped in a phosphate buffer solution containing sodium dodecyl sulfate above critical micellar concentration (CMC). The CPM and AP were separated from each other and from plasma proteins within 5 min. The calibration line indicated good linearity in the concentration range over 10 to 300 μg/ml.

KEYWORDS —— micellar electrokinetic capillary chromatography; cefpiramide; SDS; plasma sample; solubilization; zone electrophoresis; electroosmosis; β-lactam antibiotics

Electrokinetic chromatography, first introduced by Terabe et al.,[1] is in a category of free zone electrophoresis combined with chromatographic partition. It uses an open tubular capillary and an ionic surfactant solution with concentration above CMC. The technology of this method is essentially based on the capillary zone electrophoresis (CZE) developed by Mikkers,[2] Jorgenson[3] and Tsuda.[4] When ionic surfactant is present in the supporting electrolyte solution for CZE, the micelles of the surfactant (e.g. sodium dodecyl sulfate (SDS)) serve as a stationary phase which solubilizes sample substances and moves slowly, so that the separation is achieved depending on the degree of solubilization in cooperation with electroosmotic flow and electrophoretic migration of the micelles. So this method allows the samples even with no electric charge to be eluted with retention times between those of entirely solubilized substance (longest retention time) and

insolubilized substance (shortest retention time). Since the electroosmosis produces an uniform profile of flow velocity (i.e. plug flow), the sharp separation with extremely high theoretical plate number up to some hundred thousands of plates can be attained in a relatively short period of time. Theoretical explanations[5] and experimental characterization[6,7] were described previously with demonstrations of the separation of standard mixtures of PTH-amino acids,[8] chlorinated phenols,[9] oligonucleotides,[10] purines,[11] pyridoxines[12] and determination of ingredients of antipyretic analgesic preparations.[13] However, further investigations are necessary to make this method fit for practical uses such as monitoring of drug levels in biological samples. This communication briefly reports the utility of this method for the separation and determination of cefpiramide in human plasma.

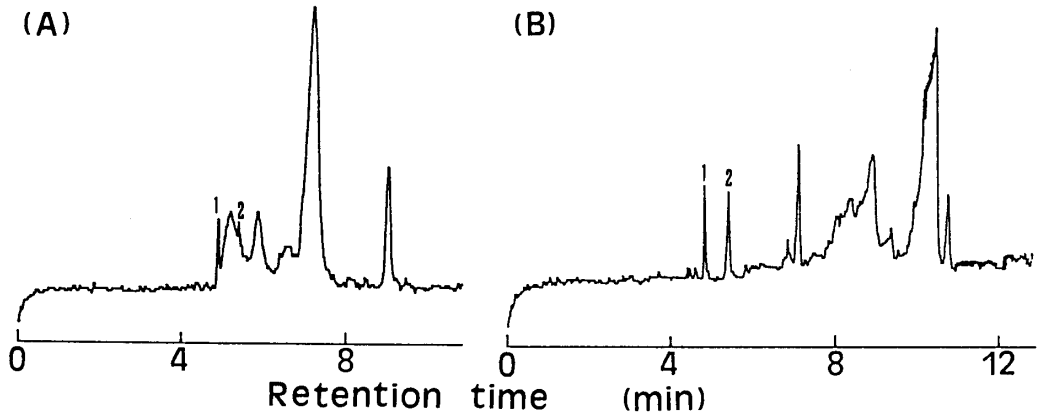

Fig. 1. Separation of Cefpiramide (Peak 2) and Antipyrine (Peak 1) in Human Plasma Achieved in (A) Phosphate Buffer Solution and (B) Phosphate Buffer Solution Containing 10 mM SDS

A fused silica capillary tube (60 cm x 50 μm i.d., Scientific Glass Engineering Inc.) was filled with a phosphate buffer solution (pH = 8.0, I = 0.05). Human plasma spiked with CPM (150 μg/ml) and AP (100 μg/ml) was introduced into the capillary by siphoning for 10 s. The siphoning was done by dipping one end of the capillary in the sample solution whose level was raised 5 cm higher than the other end which was dipped in the buffer solution. After the sample was introduced, the end of the capillary was quickly transferred to the buffer solution. The buffer solution at each end of the capillary was contained in a small glass beaker capped with a silicon-rubber stopper having two small-bore holes, one for the insertion of the capillary and the other for the platinum wire electrode. High voltage was applied to the electrodes (15 kV at the end of the capillary where the sample was introduced and 0 V at the other end) using a dc power supplier (Model HSR24-P, Matsusada Precision Device Co., Kusatsu, Shiga, Japan). The value of electric current was ca. 11 μ-Ampere. About a 2-mm length of the capillary 20 cm distant from the anodic end was made transparent by carefully burning away the polymer coating. This was used as an on-column cell for UV-detection. The effective length of the capillary between the positions of sample introduction and detection was 40 cm. The compounds were detected at 280 nm with a UV absorption detector (UVIDEC 100-III, Jasco, Tokyo, Japan) operating at 25 °C in a temperature-controlled chamber.

Fig. 1 (A) shows the separation achieved by direct introduction of human plasma spiked with cefpiramide (CPM) and antipyrine (AP, internal standard), where SDS was not involved in the buffer solution. The operating conditions are given in the legend of Fig. 1. The peaks of CPM and AP were well separated from each other, but they overlapped those of endogenous plasma components. The complete separation from these interfering peaks was not attained by changing the operating conditions except for the addition of SDS. Fig. 1 (B) shows the improved separation of the same plasma sample when 10 mM SDS was added to the buffer solution used in Fig. 1 (A). The retention times of the plasma proteins were selectively prolonged and the drug peaks were separated within 5 min. Although it is hard to explain the precise reason for such clear separation, perhaps the plasma proteins solubilized by SDS had a strongly negative charge and therefore tended to migrate toward the cathodic end of the capillary (where the sample was introduced), resisting the electroosmotic flow which moves toward the anodic end (where the detection was carried out) faster than the electrophoretic migration. This may have affected the plasma proteins more strongly than the CPM and AP, resulting in retarded elution of the interfering peaks. To avoid the consequent lengthened analysis time, elution was discontinued just after the emergence of the CPM peak and the plasma proteins were swept out of the capillary by injecting 0.1 M NaOH using a 50 μl microsyringe. This procedure also had the merit of regenerating the surface potential of the fused silica capillary tube and gave the reproducible results. The calibration line for CPM obtained in this manner with use of AP as an internal standard indicated good linearity ($r = 0.998$) over the concentration range from 10 to 300 μg/ml of plasma solution. The limit of detection was 5 μg/ml at S/N 3. This calibration line was almost identical with that obtained by introducing a buffer solution containing CPM and AP in the same range of concentration as above. This means that CPM bound to plasma protein was completely released during the processes of separation and the total plasma concentration was quantified. However, since CPM is strongly bound to plasma proteins[14] and its release involves an additional kinetic process, the theoretical plate number measured from the peak of CPM applied as the plasma solution was about 30% of that applied as the buffer solution ($N = 197500/m$, $HETP = 5$ μm). In contrast, theoretical plate number measured from the peak of AP ($N = 152500/m$, $HETP = 6.5$ μm) was not lowered as much as above because of its weaker protein binding[15]. As for the reproducibility, the coefficient of variation (CV) for the retention time of CPM in plasma sample was <5% ($n = 10$), while that in the buffer solution was <1.5% ($n = 10$). The CV value for the peak-area ratio (CPM/AP)

was 3.3% for the buffer solution and 3.8% for the plasma sample at a CPM level of 100 μg/ml (n = 7). These values are not necessarily satisfactory, but can be improved by further modification of the operating conditions with respect to depression of adsorption of sample materials onto the inside surface of the capillary, rigorous control of temperature, and reproducible introduction of the sample solution. Compared with conventional HPLC, this method has the merit of rapid separation with extremely high efficiency (10 times or much higher plate number than the usual HPLC) following direct introduction of a very small volume (ca. 10 nl) of plasma sample without pretreatment. A more detailed investigation is in process.

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