Journal of Chromatography, 590 (1992) 364–368
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 802

Short Communication

Determination of cefixime and its metabolites by high-performance capillary electrophoresis

Susumu Honda*

Faculty of Pharmaceutical Sciences and Pharmaceutical Research and Technology Institute, Kinki University, Kowakae, Higashi-osaka (Japan)

Atsushi Taga and Kazuaki Kakehi

Faculty of Pharmaceutical Sciences, Kinki University, Kowakae, Higashi-osaka (Japan)

Shigetaka Koda and Yoshihiko Okamoto

Research Laboratories, Fujisawa Pharmaceutical Company, Kashima, Yodogawa-ku, Osaka (Japan)

(First received July 4th, 1991; revised manuscript received October 14th, 1991)

ABSTRACT

Cefixime (CX), an oral cephalosporin antibiotic, and its metabolites in human digestive organs were separated by various modes of high-performance capillary electrophoresis. The zone electrophoresis mode in phosphate buffer (pH 6.8) containing 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulphonate gave the best separation, permitting the complete resolution of CX and all of five metabolites. On the other hand, the plain zone electrophoresis mode in phosphate buffer (pH 6.8) offered a simple procedure for the direct determination of urinary CX concentration using intact urine samples.

INTRODUCTION

High-performance capillary electrophoresis (HPCE) is now widely applied in analyses of biological substances, especially oligo- and polynucleotides (*e.g.*, [1]), and applications in other fields, including drugs and metabolites, are developing.

Cefixime, (6*R*,7*R*)-7-[(*Z*)-2-(2-amino-4-thiazolyl)-2-(carboxymethoxyimino)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, is a cephalosporin antibiotic for oral administration, having a broad spectrum for Gram-negative bacteria. It is known to be metabolized in human digestive organs to give compounds as shown in Fig. 1, by the action of bacterial enzymes.

CX and its metabolites, M1–M5, have a common characteristic feature that they all have carboxyl groups(s), and hence are hydrophilic. For this reason, they cannot be extracted from biological fluids by solvent extraction. From an analytical viewpoint, the strong hydrophilicity also hampers their separation by liquid chromatography in the direct partitioning mode. Separation was barely achieved by reversed-phase partitioning with ion pairing to the tetrabutylammonium ion in the mobile phase [2]. In this paper, we propose an alternative method

0021-9673/92/$05.00

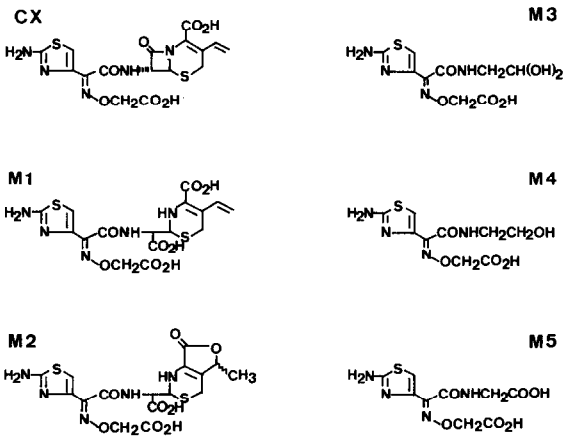

Fig. 1. Structures of cefixime (CX) and its metabolites (M1–M5).

based on high-performance capillary electrophoresis (HPCE).

EXPERIMENTAL

Chemicals

CX and its metabolites were prepared by Fujisawa Pharmaceutical (Osaka, Japan) and used as received. 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulphonate (CPDAPS) and 1,5-dimethyl-1,5-diazaundecamethylene polymethobromide (polybrene or hexadimethrine bromide) were obtained from Sigma (St. Louis, MO, USA) and Aldrich (Milwaukee, WI, USA), respectively. All other chemicals were purchased from Wako (Osaka, Japan).

HPCE

HPCE was performed by using a Model 270 A capillary electrophoresis system (Applied Biosystems, San Jose, CA, USA), equipped with an automated vacuum injector, a UV detector, a thermostated bath and a data processor. A capillary tube of fused silica (50 μm I.D., 375 μm O.D.) was used for all modes of HPCE, and detection was carried out in the on-column mode at the 22-cm position from the outlet. At this position of the tube the polyimide coating was removed and the transparent portion was fixed on the detector block. The compositions and pH values of the carriers are given in the figure captions. The electric current was *ca.* 30 μA in all systems. Sample solutions were introduced into the tube by suction under a weak vacuum for 1.5 s.

RESULTS AND DISCUSSION

Separation modes

The basic mode of separation in HPCE is zone electrophoresis, but other modes can be realized simply by dissolving additives in the carrier. In this work the separation of CX and its metabolites was examined using the zone electrophoresis and electrokinetic modes under various conditions.

Zone electrophoresis mode. In the zone electrophoresis mode separation is based on the difference in electric charge relative to molecular size among the sample components. The magnitude of the electric charge is dependent on the number of carboxyl groups. It also depends on the pH of the carrier, because the dissociation of the carboxyl group is controlled by pH. Therefore, separations at various pH values were compared using 50 m*M* phosphate buffer. The best separation was achieved at pH 6.8. Fig. 2 shows the electropherogram obtained under these conditions utilizing a capillary tube of fused silica.

A mixture of CX and its metabolites was introduced from the anodic end of the tube. Electroosmotic flow was toward the cathode and the electrophoretic attraction of the sample components to the anode was at lower velocities than that of electroosmosis. The metabolites except M3 and M4 were completely separated from each other; the peaks of M3 and M4 partially overlapped, because their structures are only slightly different from each other (in the side-chain at the 5-position of the thiazole ring). Metabolite M2 was unfortunately unresolved from CX. CA denotes cinnamic acid added as an internal standard, which was well separated from CX and the metabolites.

Suppression of electroosmotic flow by addition of hydroxypropylcellulose at a concentration of 0.02% and introduction of the sample from the cathodic end of the tube resulted in the appearance of peaks in the reverse order, but the separations of the M3–M4 and M2–CX pairs were not improved (Fig. 3).

Addition of CPDAPS to 20 m*M* phosphate buffer (pH 6.8) at a low concentration of 0.15% (w/v)

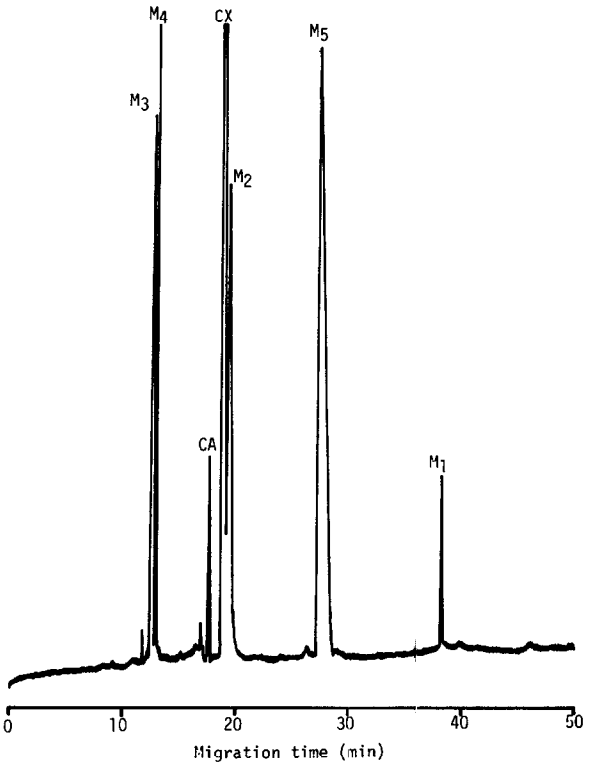

Fig. 2. Separation of CX and its metabolites by plain zone electrophoresis with electroosmotic flow. Capillary, fused silica (72 cm × 50 μm I.D.); carrier, 50 m*M* phosphate buffer (pH 6.8); applied voltage, 15 kV; detection, UV absorption at 280 nm; sample solution, aqueous solution containing CX, M1, M2, M3, M4, M5 and cinnamic acid (CA) (internal standard); sample concentration, $1.0 \cdot 10^{-3}$ *M* (CX, M1, M2, M3, and M4), $1.0 \cdot 10^{-4}$ *M* (M5) and $5.0 \cdot 10^{-4}$ *M* (CA). The sample solution was introduced from the anodic end of the tube.

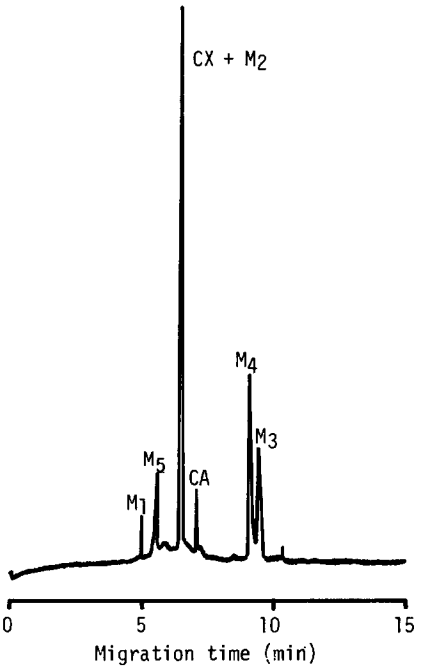

Fig. 3. Separation of CX and its metabolites by plain zone electrophoresis without electroosmotic flow. Capillary, fused silica (68 cm × 50 μm I.D.); carrier, 20 m*M* phosphate buffer (pH 6.8) containing hydroxypropylcellulose (0.02%, w/v); applied voltage, 20 kV. Other conditions as in Fig. 2. The sample solution was introduced from the cathodic end of the tube.

caused a drastic change in the electropherogram; all components gave their peaks in 15 min and separations of the M3–M4 and M2–CX pairs were not so good. In addition, the migration time of M1 became especially shorter, and as a result the peaks of M1 and M5 partially overlapped (electropherogram not shown). However, addition of methanol to this system at a concentration of 20% (v/v) resulted in retardation of the peaks and an improvement in the separation of the M3–M4, M2–CX and M1–M5 pairs compared with those in the carrier not containing methanol (Fig. 4).

In the zone electrophoresis mode, addition of methanol generally causes retardation and improved separation of peaks, because the degree of hydration of the component ions is reduced and accordingly the effective sizes of the ions are decreased (*e.g.*, ref. 5). The phenomenon observed in Fig. 4 is

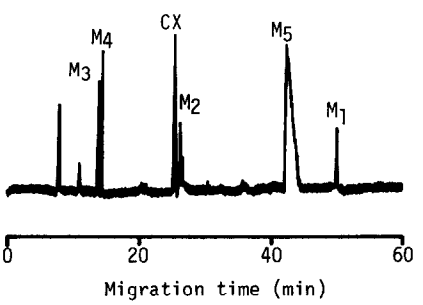

Fig. 4. Separation of CX and its metabolites in the CPDACS–methanol-containing carrier. Capillary, fused silica (75 cm × 50 μm I.D.); carrier, 20 m*M* phosphate buffer (pH 6.8) containing CPDAPS (0.15%, w/v) and methanol (20%, v/v); applied voltage, 30 kV. Other conditions as in Fig. 2. The sample solution was introduced from the anodic end of the tube.

mainly due to this effect, but the effect of addition of CPDAPS cannot be neglected for the separation of the M2–CX pair, as the resolution of this pair in phosphate buffer containing both CPDAPS and methanol was 1.75, whereas that in phosphate buffer containing only methanol was 1.35. The effect of CPDAPS was not predominent, but this amphoteric detergent played a subsidiary role. It seems that the cholamidopropyl part of the CPDAPS molecule interacted weakly with these compounds, and the slight difference in the magnitude of such an interaction between M2 and CX caused an improvement in their separation. The resolutions of the M3–M4 and M1–M5 pairs were much improved by addition of methonol but was not affected by addition of CPDAPS.

Electrokinetic chromatography mode. Terabe *et al.* [3] initiated micellar electrokinetic chromatography by addition of sodium dodecyl sulphate (SDS), an anionic surfactant, to the carrier. This mode permits separation based on solubilization of sample components to moving SDS micelles. However, no solubilization occurred in this work when SDS was added to 20 m*M* phosphate buffer (pH 6.8) at concentrations of 10—50 m*M*, as all components were only slightly hydrophobic under these conditions. The migration times of all these components were gradually increased with increasing SDS concentration, but the pattern of the electropherogram was the same as that in Fig. 2. The separations of the M3–M4 and M2–CX pairs were worse than those in the plain CZE mode (Fig. 2).

On the other hand, Terabe and Isemura [4] studied the separation of highly acidic compounds in a carrier containing polybrene, a basic polymer, and designated this mode ion-exchange electrokinetic chromatography. In this mode, the direction of electroosmotic flow is reversed owing to the change in sign of the electric charge on the capillary inner wall, caused by adsorption of this basic polymer. When this mode was applied in the present instance (Fig. 5), the migration order (CA, M4, M1 + M3, M5, M2, CX, in order of increasing migration times) was different from that in Fig. 2 (M3 + M4, CA, M2 + CX, M5, M1).

If a higher electronegativity of a component leads to a greater ability to bind to this basic polymer, as in ion-exchange chromatography, then components having larger numbers of carboxyl groups should give longer migration times. However, the results, especially the short migration time of M2, which has the largest number of carboxyl groups (three), were inconsistent with this prediction. There might be an unknown mechanism other than ion exchange operative in this system. In this mode the separation of M2 and CX was better than in the plain zone electrophoresis and micellar electrokinetic chromatography modes, but the separation of M1 and M3 was worse.

Overall, zone electrophoresis in the CPDAPS-containing carrier gave the best separation, permitting almost complete separation of CX and its metabolites.

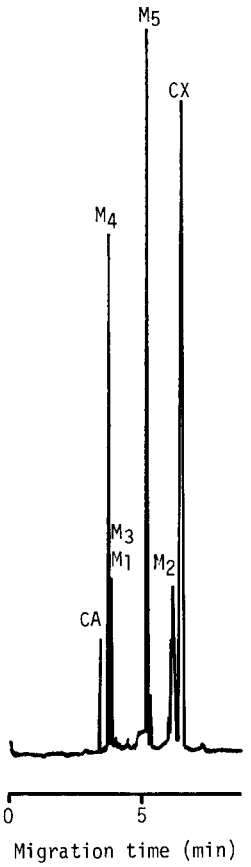

Fig. 5. Separation of CX and its metabolites in the polybrene-containing carrier. Capillary, fused silica (72 cm × 50 μm I.D.); carrier, 20 m*M* phosphate buffer (pH 6.8) containing polybrene (0.5%, w/v); applied voltage, 20 kV. Other conditions as in Fig. 2. The sample solution was introduced from the cathodic end of the tube.

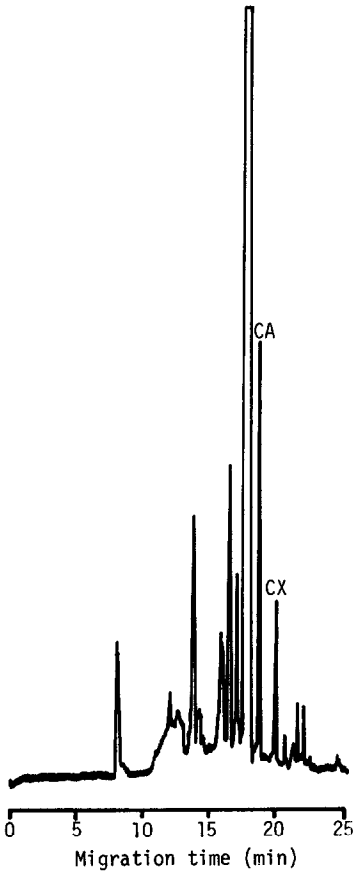

Fig. 6. Analysis of human urine spiked with CX and CA by plain zone electrophoresis. Capillary, fused silica (75 cm × 50 μm I.D.); applied voltage, 20 kV; detection, UV absorption at 295 nm. Concentration: CX, 30.0 μg/ml; CA, 85.1 μg/ml. Other conditions as in Fig. 1. The sample solution was introduced from the anodic end of the tube.

Determination of urinary CX

Orally administered CX is known to be absorbed from the intestine, transported into the circulatory system and excreted into urine almost without being metabolized. Therefore, the determination of the CX concentration in urine provides useful information on whether the CX level is properly controlled or not in the body.

The plain zone electrophoresis mode using 50 m*M* phosphate buffer (pH 6.8) as the carrier, applied to a urine specimen spiked with CX, together with CA as an internal standard, gave the electrophoretogram shown in Fig. 6. The peaks of both CX and CA were well separated from those of inherent urinary components.

Plots of the relative peak response of CX to CA (8.51 μg/ml) against CX concentration in urine gave a straight line almost passing through the origin ($y = 0.0166x - 0.0414$, $r = 0.996$) at least over the range 10–60 μg/ml, which covers the therapeutic range. The relative standard deviations ($n = 7$) at the 30 and 60 μg/ml levels were 3.6% and 2.9%, respectively. This method is simple, permitting the direct determination urinary CX.

REFERENCES

1 A. S. Cohen, D. R. Najarian, A. Paulus, A. Guttman, J. A. Smith and B. L. Karger, *Proc. Natl. Acad. Sci. U.S.A.*, 85 (1988) 9660.
2 Y. Namiki, T. Tanabe, T. Kobayashi, J. Tanabe, Y. Okimura, S. Koda and Y. Morimoto, *J. Pharm. Sci.*, 76 (1987) 208.
3 S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya and T. Ando, *Anal. Chem.*, 56 (1984) 111.
4 S. Terabe and T. Isemura, *Anal. Chem.*, 62 (1989) 650.
5 S. Fujiwara and S. Honda, *Anal. Chem.*, 59 (1987) 487.