Determination of Antibacterial Flomoxef in Serum by Capillary Electrophoresis

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Abstract

A determination method of flomoxef (FMOX) concentration in serum by capillary electrophoresis is developed. Serum samples are extracted with acetonitrile. After pretreatment, they are separated in a fused-silica capillary tube with a 25mM borate buffer (pH 10.0) as a running buffer that contains 50mM sodium dodecyl sulfate. The FMOX and acetaminophen (internal standard) are detected by UV absorbance at 200 nm. Linearity (0–200 mg/L) is good, and the minimum limit of detection is 1.0 mg/L (S/N = 3). The relative standard deviations of intra- and interassay variability are 1.60–4.78% and 2.10–3.31%, respectively, and the recovery rate is 84–98%. This method can be used for determination of FMOX concentration in serum.

Introduction

Flomoxef (FMOX) is an oxacephem antibiotic (Figure 1) developed by Shionogi Research Laboratories (Osaka, Japan), which exhibits antimicrobial activity mainly against gram-negative microorganisms. It also has a wide spectrum of antimicrobial activity and excellent safety against general germs, and these properties often make this drug a first-choice antimicrobial agent and usable even in empiric therapy (1). FMOX is hardly metabolized in a living organism and mostly excreted into urine as an unchanged drug. However, in the case of a patient with renal dysfunction, because the disappearance half-life of this drug is prolonged, intervals between administrations as well as a dosage suitable for the renal function need to be established. Additionally, because FMOX has caused serious side effects in some cases, as seen with other antimicrobial agents, this drug requires monitoring of its concentration in blood (2). Kimura et al. (3), using a bioassay method, and Konaka et al. (4), using high-performance liquid chromatography (HPLC), determined FMOX concentration in human plasma and urine. Because FMOX has UV region absorption as well as ionic functional groups in itself, we paid attention to the possibility of its separation by capillary electrophoresis (CE) with a fused-silica capillary tube of high durability and its economical determination with the samples and electrophoresis buffer of small volumes. The purpose of this research was to develop a new method for determining FMOX concentration in serum by CE.

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Experimental

Antimicrobial agents

FMOX sodium was kindly provided by Shionogi Pharmaceutical (Osaka, Japan). Cefazolin, cefmetazole, cefoperazone, cefotaxime, and ceftriaxone (Sigma, St. Louis, MO) were used.

Reagents

Sodium tetraborate decahydrate, sodium hydroxide (0.1N), sodium dodecyl sulfate (SDS), and acetonitrile (Wako Pure Chemicals, Osaka, Japan) were used. The acetaminophen used as an internal standard (IS) was made by Sigma.

Preparation of standard solutions

An FMOX stock standard solution (1.0 g/L) was prepared with distilled water and then diluted with appropriate volumes of distilled water into working standard solutions of various concentrations. The IS solution was adjusted with acetonitrile so that it became 1.75 mg/L.

Sample preparation

A 0.1-mL serum sample and 0.4 mL of acetonitrile that contained the IS were mixed together, stirred, and centrifuged at

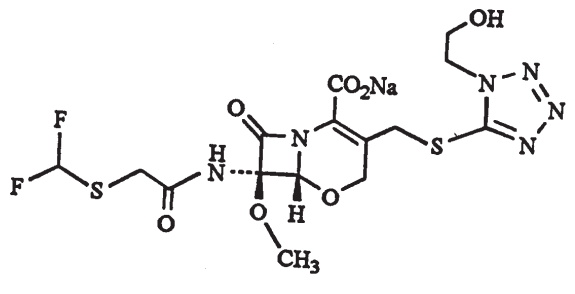

Figure 1. Structure of FMOX sodium.

12,000 g for 2 min. Its supernatant was then poured into another polypropylene tube, evaporated to dryness at room temperature under reduced pressure using a centrifugal concentrator (Taitec, Tokyo, Japan), and redissolved in 0.05–0.1 mL of distilled water to be used as CE samples.

CE instruments and initial electrophoresis conditions

A P/ACE system MDQ CE made by Beckman Coulter (Fullerton, CA), an untreated fused-silica capillary tube of a 670-mm length (effective length of 500 mm), and a 75-µm bore were used. A 25mM borate buffer containing 50mM SDS was used as a running buffer, which was mixed with sodium tetraborate decahydrate and sodium hydroxide (0.1N) to be adjusted to pH 10.0. The running buffer was further passed through a disposable filter of 0.45 µm (Millipore, Bedford, MA) and de-aired ultrasonically for 5 min before actual use. The capillary tube was rinsed with sodium hydroxide (0.1N) for 15 min (20 psi) without fail when analysis was started. Additionally, it was rinsed with sodium hydroxide (0.1N) for 3 min, then with distilled water for 3 min, and further conditioned with the running buffer for 5 min (20 psi) before each run. The sampling time was 8 s (0.5 psi), applied voltage was 25 kV (normal polarity) (25ºC), and detection was performed at 200 nm.

Linearity

Measurements were taken for standard serum samples of 6.3, 12.5, 25, 50, 100, and 200 mg/L, which were prepared by adding the FMOX standard solutions of various concentrations to normal pooled serum. FMOX peak height ratios versus IS were then plotted against FMOX concentrations to obtain linear regression.

Lowest limit of quantitation

The lowest concentration of the serum sample added with the FMOX standard solution, measurable when the signal-to-noise (S/N) ratio against background noise was 3, was determined.

Precision

For determining intra-assay variability, FMOX-added serum samples of five different concentrations were prepared, and each sample was consecutively measured five times. For determining interassay variability, FMOX-added serum samples of three different concentrations were prepared, and each sample was measured two times a day in a consecutive manner for five consecutive days. The samples were stored at –20ºC during the whole procedure.

Recovery

Serum samples prepared by adding the FMOX standard solutions of various concentrations to blank serum were measured three times in a consecutive manner, respectively, so that the average relative standard deviation (RSD) and recovery rate could be calculated.

Selectivity

In order to evaluate interference with the determination from endogenous substances in the serum, the electropherograms of blank serum and these substances were compared. Other cephem antimicrobial agents (the concentration for evaluation of all of them was 2.0 g/L) that might be used together with FMOX were determined so that influence of them on the determination of this drug could be evaluated by comparing migration times of those agents with that of FMOX.

Results

Figure 2 shows the electropherograms of blank serum and the separated FMOX peak. Specific analysis of FMOX and IS was achieved because there was no interference on the determination from endogenous substances in the serum. Linearity (0–200 mg/L) was obtained when $r = 0.9999$ and y (FMOX peak height/IS peak height) $= 0.0100 \times$ (FMOX concentration) $- 0.00355$. The minimum limit of detection (LOD) was 1.0 mg/L at an S/N ratio of 3. Evaluation results of intra- and interassay variability are given in Table I. Results of the recovery rate test are shown in Table II. Elution behaviors of other cephem antimicrobial agents under the determination conditions of this method were evaluated, and the ratio of the migration time of each agent to that of

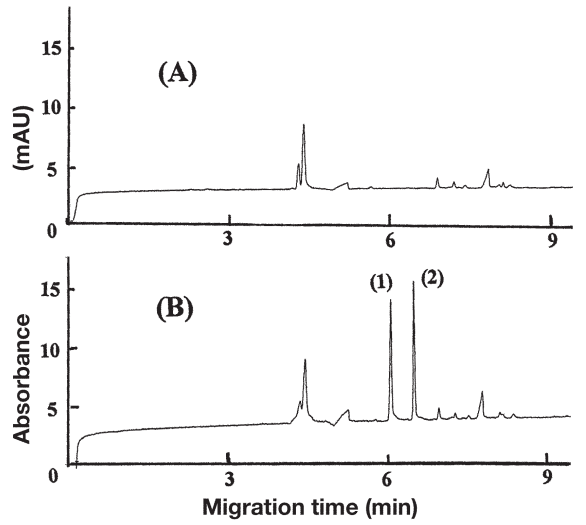

Figure 2. Typical chromatograms of a blank serum (A) and a serum standard spiked with FMOX (50.0 mg/L) (B). (1) FMOX and (2) IS.

Table I. Intra- and Interassay Variabilities of Spiked Serum Samples for FMOX Assay

Mean value (mg/L)	SD* (mg/L)	RSD (%)
Intra-assay variability ($n = 5$)		
6.9	0.33	4.78
16.0	0.256	1.60
28.8	0.531	1.84
66.2	2.19	3.31
120.9	4.380	3.62
Interassay variability (5 days)		
10.0	0.210	2.10
21.1	0.699	3.31
51.0	1.14	2.24

* SD, standard deviation.

Table II. Absolute Recoveries for FMOX Assay*

Theoretical concentration (mg/L)	Mean value (mg/L)	SD† (mg/L)	RSD (%)	Recovery (%)
2.5	2.1	0.082	3.90	84.0
5.0	4.5	0.094	2.09	90.0
12.5	10.5	0.591	5.63	84.0
25.0	23.1	0.216	0.935	92.4
50.0	46.9	2.18	4.65	93.8
100.0	97.8	1.08	1.10	97.8

* $n = 3$.
† SD, standard deviation.

FMOX [relative migration time (RMT)] was calculated. The following results were obtained: cefazolin, 1.071; cefmetazole, 1.028; cefoperazone, 1.177; cefotaxime, 1.009; and ceftriaxone, 1.382. The RMT of the IS was 1.084. All of the other antimicrobial agents evaluated did not interfere with the determination of either FMOX or IS.

Discussion

Separation analysis methods are most suitable for precise determination of FMOX concentration in serum. Of these methods, HPLC and CE are especially useful, and both are widely used (5). The determination of antibacterial drugs has been achieved using HPLC.

HPLC is an easy analytical method, but an HPLC column is a little expensive and comparatively low in durability. However, a capillary tube used for this method rarely has problems such as blinding or deterioration, is highly durable, and requires extremely tiny volumes of a run buffer and samples. Recently, CE has been recognized as an alternative to HPLC because CE requires less sample, and solvent waste is negligible. Attention was given to the advantages of a fused-silica capillary tube used for CE over a separation column packed with material used for HPLC. It offered a higher theoretical plate number as well as higher durability and enabled a determination with samples of microquantities, therefore we used it for the FMOX determination. Using serum without pretreatment as a CE sample is simple and extremely efficient (6,7), and therefore it can be applied to the cases in which detection is performed in a range with a relatively high wavelength measurement and in which the determined concentration is equal to or higher than approximately 5 mg/L (8). First, we attempted the determination of FMOX with the method using serum without pretreatment at lower wavelengths, but this was not successful because of interference from endogenous substances in serum. The determination turned out to be possible without substantial interference from endogenous substances absorbing at higher wavelengths and without pretreatment of serum at higher wavelengths, but the analysis was to be impossible because the limits of quantitation became lower. In most cases, proteins that account for a large part of serum have serious influence on the determination (9). Acetonitrile, because of its high deproteinization efficiency, is widely used for general purposes. Shihabi et al. (10) reported a theophylline analysis in which they deproteinized serum by adding acetonitrile one and half times as much as the serum and used its supernatant as a CE sample. We performed the same pretreatment, and detection was conducted at 200 nm, but FMOX determination was unsuccessful because of interference from impurities in the serum. Brunner et al. (11) reported an antipyrine determination using organic solvent extraction. That is, they evaporated to dryness a liquid extracted with acetonitrile, redissolved it in distilled water, and used it as a CE sample. This method, in which the CE sample is aqueous, allows micellar electrokinetic capillary chromatography to easily exhibit its effect of increased separation efficiency. It also allows enrichment to be performed through the adjustment of the redissolved liquid volume, which contributes to the increase of measurement sensitivity. However, an appropriate IS needs to be selected and used because the fluctuation of the recovery rate is expected during the pretreatment. We performed extraction with acetonitrile four times as much as the serum to be used. It was confirmed that this method allowed no interference from endogenous substances, and it enabled measurement even with a low wavelength. Additionally, it was observed that acetaminophen, which showed slower migration time than FMOX, was most suitable as an IS. In order to increase ionic strength of the substance to be measured and obtain a higher resolution factor, the type of electrophoresis buffer to be used and its pH value are two major factors to consider (12,13). The higher the pH value of the electrophoresis buffer and the SDS concentration (the maximum is 300mM) are, a higher resolution factor can be obtained. Although, in this case a load current value increase resulted in a lower determination precision. Sensitivity increases with greater sampling time, but there is a threshold value according to the capillary tube capacity. Thus, with this method, optimum analytical conditions for FMOX were designed.

Konaka et al. (4) conducted a determination of FMOX in serum with HPLC in which they reported an elution time of 15 min and a minimum LOD of 0.5 mg/L (S/N = 3). Their method offers a precision of almost the same level as our CE method, although the elution time of this method was shorter (6 min).

Conclusion

Volumes of the running buffer and samples used for this method were of microquantities, and the capillary tube could exhibit extremely high durability when it was rinsed periodically with methanol as well as acidic and alkaline solvents, which enabled an economical analysis. Furthermore, this method allowed the determination of FMOX concentration in serum to be achieved with high precision and accuracy in a specific, simple, and fast manner.

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