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# Determination of cefuroxim levels in human serum by micellar electrokinetic capillary chromatography with direct sample injection[1]

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**Abstract**

Effective monitoring of cefuroxim levels by micellar electrokinetic capillary chromatography with direct serum injection are discussed and compared with the HPLC method. With capilliary electrophoresis (CE), in contrast to HPLC, good resolution and efficiency was demonstrated as well as low consumption of solvent and samples. The CE system was applied at 15 kV with UV detection at 274 nm using 150 mM sodium dodecyl sulfate in 20 mM sodium phosphate and borate (pH 9.0) as electrolyte. The results were seen within 12 min with efficiency approaching 182 000 theoretical plates. The coefficients of variations of migration time and peak area were less than 0.8 and 5.9%, respectively. The detection limits for quantitative determination were 0.28 μM level. Good linearity and recovery were also obtained in the range of serum levels usually encountered in clinical analysis with a correlation coefficient of $r = 0.991$ and 98–101% recovery. The monitoring of cefuroxim in human serum with micellar electrokinetic capilliary chromatography (MECC) is demonstrated. Identification of cefuroxim in human serum with MECC is demonstrated. Identification of cefuroxim was performed by characterizing the sample peak in terms of the migration time and UV spectrum. Considering the results of our study, the CE method should be highly suitable for the separation of cefuroxim in biofluids. © 1997 Elsevier Science B.V.

*Keywords:* Cefuroxim; Micellar electrokinetic capillary chromatography; Biofluids

## 1. Introduction

The more efficient therapeutic application of many drugs has given rise to a need for reliable analytical procedures to measure their serum and plasma levels. These are routinely used high performance liquid chromatography (HPLC) and immunoassays in many clinical laboratories [1,2]. With the HPLC technique a side variety of drugs

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and metabolites can be analyzed but it still requires a relatively high level of analytical skill and complex pretreatment of the samples. The advantage of capillary electrophoresis (CE) over HPLC are simplicity and low cost of the operation, because no packed column, pumps and mobile-phase gradient are used. Many of the common problems associated with HPLC such as high pressure, solvent leakage, column equilibration and the high cost of columns are eliminated. Also, changing buffers for different tests is much easier in CE which needs no lengthy column equilibration. Immunological methods analyze only one drug at a time, do not measure metabolites and in some cases are subject to problems with cross-relative interference. Since CE was introduced as a new clinical tool for serum, spinal fluid and urinary analytical separations [3], many clinical investigators have recently applied micellar electrokinetic capillary chromatography (MECC) for drug monitoring. MECC is a new type of separation method based on micellar partitioning of the solute and electrophoretic migration of the micelle. MECC has many attractive advantages in additions to the capability of electrophoretic separation of electrically neutral substances [4]. Its main advantages over conventional methods are ability to separate both neutral and charged compounds in a single run as well as the possibility of direct application of proteinaceous material. Serum samples with sodium dodecyl sulfate (SDS) as protein solubilizer can be directly injected and drugs can be analyzed, provided they elute before the proteins and they are present in a few μg $ml^{-1}$ when on-column UV absorption detection is employed. Many investigators have successfully achieved the determination of drugs in serum and plasma by MECC with a direct sample injection method, similarly to micellar HPLC [5–8]. Cefuroxim, one of the second-generation cephalosporin antibiotics, has been used widely and, in serum, has been analyzed previously by HPLC after pretreatment [9,10]. This study was undertaken to determination cefuroxim in human serum by MECC with direct serum injection and to compare it with the HPLC method.

## 2. Experimental

### *2.1. Reagents and the sample preparation*

Cefuroxim, cefrazidime and cephalexin were supplied by Glaxo Korea (Seoul, South Korea) and cefoperazone, caphradine and cefoxitin were supplied by Shinpoong Pharmaceutical (Seoul, South Korea). SDS was purchased from BioRad (Hercules, CA) and sodium phosphate and sodium borate were purchased from Merck (Darmstadt, Germany). Methanol and water were purchased from J.T. Baker (Phillipsburg, NJ). Serum samples were supplied by Yonsei University Hospital (Seoul, South Korea). Sera containing cefuroxime were obtained after patients were given an injection of 750 mg $ml^{-1}$ and then prepared by centrifugation at $1350 \times g$ for 10 min. All sera were stored at −20°C.

The electrolyte was composed of 150 mM SDS, 20 mM sodium phosphate and 20 mM sodium borate and adjusting the pH to 9.0 with 0.1 M sodium phosphate. The electrolyte was filtered through a 0.45 μm filter. Standard stock solutions of cefuroxim (1 mg $ml^{-1}$) for calibration were prepared in double distilled water to a certain concentration. Drug concentrations in human serum spiked with standard solution were determined. The patient's sera were simply defrosted vortexed and filtered through a 0.45 μm filter before injection.

### *2.2. Apparatus and method*

A CE system equipped with a P/ACE 5500 diode array detector, automatic injector, a fluid cooled column cartridge and a system Gold data station (Beckman, Fullerton, CA) was used in this study. All runs were carried out at 20°C. The electrolyte was passed through 0.2 μm nylon filters and degassed prior to use. The capillary inlet and outlet vial were replenished after every run. Injections were made using the pressure mode for 5 s at $3.45 \times 10^{-3}$ MPa. Detection was performed at a wavelength of UV 274 nm. A fused silica capillary (57 cm long × 75 μm i.d.) was rinsed with water and filled with electrolyte for 5 min prior to sample injection. If drastic

drifts in electrophoretic current and/or migration times were observed the capillary was treated between the different sample injections by rinsing with 0.1 M NaOH solution for 15 min followed by 10 min rinses with deionized water, methanol, deionized water again and the running buffer. During the runs the capillary was applied with a voltage of 15 kV (253 V $cm^{-1}$), and the current was measured at approximately 131 μA.

HPLC were performed using a model LC-6A pump (Shimazu, Tokyo, Japan) and a model SPD-6 AV detector (Shimadzu). The column (125 mm × 4.6 mm i.d.) was a Lichrospher 100 RP-18 (Shimazu). The mobile phase consisted of a mixture of 2.5 mM sodium phosphate and methanol (40:9, v/v, pH 5.6). The flow rate was 1.0 ml $min^{-1}$ at 25°C.

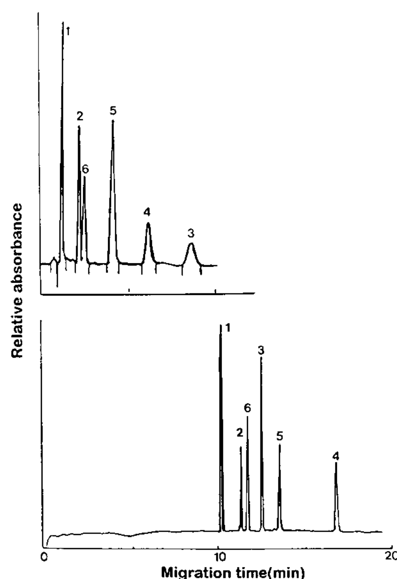


Fig. 1. HPLC chromatogram (top) and CE electropherogram (bottom) of cefalosporin antibiotics. Peak: 1, ceftazidine, 2, cefuroxime, 3, cefoperazone, 4, cephardine, 5, cephalexin, 6, cefoxitin. HPLC conditions: mobile phase, 2.5 mM sodium phosphate and methanol (40:9, v/v); flow rate, 1 ml $min^{-1}$; column, 125mm × 4.6 mm Lichrospher 100 RP-18; temperature, 20°C; detection wavelength, 254 nm; injection, 50 μl. CE conditions: electrolyte, 150 mM SDS in 20 mM sodium phosphate and 20 mM sodium borate (pH 9.0); voltage, 15 kV; capillary; 75 μm × 57 cm fused silica; injection, pressure mode for 5 s at $3.45 \times 10^{-3}$ MPa; Temperature and detector wavelength is the same as the HPLC condition.

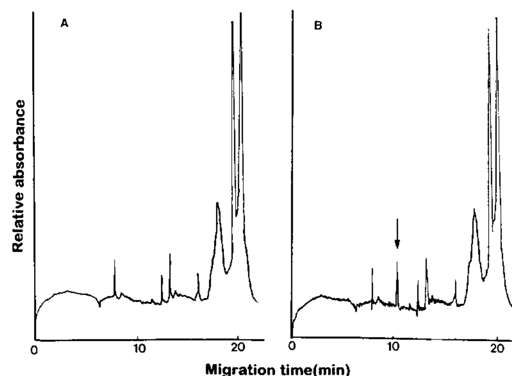


Fig. 2. Electropherogram of cefuroxim in serum (A), blank serum (B) and serum spiked with cefuroxim. Detection wavelength is 274 nm, other conditions as in Fig. 1.

## 3. Results and discussion

### *3.1. Analysis of cephalosporin antibiotics containing cefuroxim by HPLC and CE*

Currently HPLC, on reversed phase column, is the general instrumental method for the determination of cefuroxim. In a preliminary study cephalosporin antibiotics containing cefuroxim were separated by HPLC on $C_{18}$ and by MECC with SDS (Fig. 1). CE conditions were modified from H. Nishi's method [11] and the detection wavelength was 254 nm. Applied voltage was 15 kV. The retention times of cefuroxime in the HPLC chromatogram was 2.2 min and the migration time in the CE electropherogram was 11.7 min. Linearity was assessed in the concentration range 30–600 ng $ml^{-1}$ for the determination of cefuroxim by HPLC. For ten calibration graphs, the regression coefficients obtained for the determination of cefuroxim by HPLC was $r = 0.9999$ and the linearity equation was $y = 55.38 + 28.86$. The limit of detection was 0.06 μM for HPLC. Determination of cefuroxim in serum by the HPLC method in spite of fast analytical time required complex sample pretreatment such as solvent extraction, deproteinization and evaporation, etc. The number of theoretical plates obtained by HPLC was 2680 and was 182 000 by CE.

Table 1
Reproducibility of retention time and peak area of intra- and inter-day runs by MECC

| Concentration (μg $ml^{-1}$) | Migration time (min) | | | Peak area | | |
|---|---|---|---|---|---|---|
| | Mean | S.D. | CV[a] (%) | Mean | S.D. | CV (%) |
| Within-run ($n = 10$) | | | | | | |
| 10 | 11.67 | 0.03 | 0.26 | 15653.5 | 500.9 | 3.2 |
| 20 | 11.60 | 0.02 | 0.17 | 31608.6 | 1695.4 | 2.2 |
| 30 | 11.64 | 0.05 | 0.43 | 46610.1 | 1444.9 | 3.1 |
| 40 | 11.63 | 0.02 | 0.02 | 62420.9 | 1186.0 | 1.9 |
| 50 | 11.66 | 0.01 | 0.09 | 77750.6 | 1555.0 | 2.0 |
| Between-run (n = 10) | | | | | | |
| 10 | 11.78 | 0.09 | 0.76 | 15904.0 | 811.1 | 5.1 |
| 20 | 11.60 | 0.05 | 0.43 | 31100.6 | 1829.4 | 5.9 |
| 30 | 12.01 | 0.06 | 0.50 | 46902.1 | 1782.3 | 3.8 |
| 40 | 11.68 | 0.08 | 0.69 | 62712.2 | 2006.8 | 3.2 |
| 50 | 11.79 | 0.03 | 0.25 | 77760.5 | 3188.2 | 4.1 |

[a] CV, coefficient of variaitons.

Typical MECC results for standard solutions containing 20 μg $ml^{-1}$ of cefuroxim, blank serum and serum spiked with cefuroxim at fixed concentrations are shown in Fig. 2. In the electrolyte chosen for the method, the cefuroxim had$_{max}$, by stop-flow scanning, of 274 nm this was chosen as the analytical wavelength. 150 mM SDS was added to the electrolyte because interfering peaks were observed in all of the samples selected if the concentration of SDS was less than 150 mM. Serum proteins were probably solubilized by the SDS micelles because the SDS concentration in the buffer solution was above the critical micellar concentration and the migration time of plasma proteins increased with increasing concentration of SDS [12]. As indicated above, cefuroxim analysis was performed with 150 mM SDS in 20 mM borate and 20 mM sodium phosphate (pH 9.0) and the CE instrument was equipped with a 57 cm × 75 μm i.d. fused silica capillary column at 20°C. The electropherogram showed that the cefuroxim peak was well resolved on a stable base line without interfering peaks.

Table 2
Recovery of cefuroxim by MECC

| Concentration (μg $ml^{-1}$) | Recovery (mean ± S.D.) | CV (%) |
|---|---|---|
| 10 | 98.0 ± 3.0 | 3.1 |
| 20 | 98.7 ± 2.5 | 2.5 |
| 30 | 101.3 ± 1.8 | 1.8 |
| 40 | 99.3 ± 2.7 | 2.7 |
| 50 | 99.0 ± 3.1 | 3.1 |

[a] CV, coefficient of variations.

### *3.2. Reproducibility and recovery*

The precision of the peak is affected by variation in the migration time, so stable and reproducible separation conditions must be established prior to the quantitative analysis. Precision for migration time and peak area was investigated. The amount of component in the serum injected was 20 μg $ml^{-1}$. The applied voltage between the two ends of the 57 cm capillary was 15 kV. All the compounds were completely resolved within 20 min. In order to confirm the reproducibility of the migration time and peak area of cefuroxim, the standard mixture was injected repeatedly 10 times per day for three consecutive days. As shown in Table 1, the coefficients of variations (CV) of the migration time were less than 0.43% for the within-run precision and less than 0.76% for the between-run precision. Those of the peak areas were less than 3.2–5.9% both within and between runs.

The absolute analytical recovery of cefuroxim in serum was measured by spiking a known con-

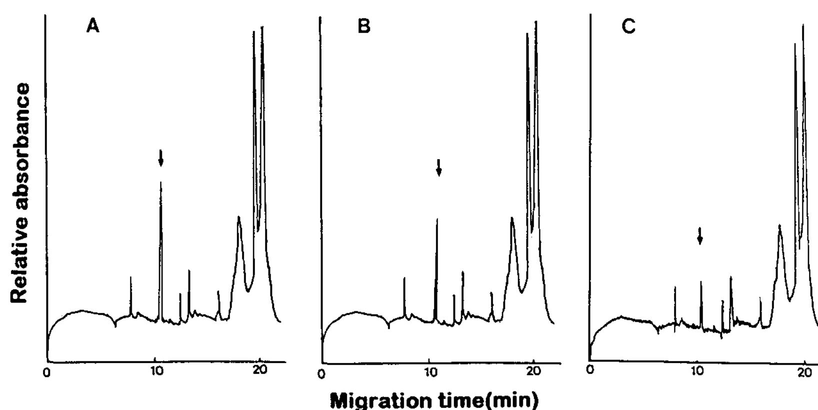


Fig. 3. Electropherogram of cefuroxim in serum. (A) 30, (B) 60 and (C) 90 min after injection (750 mg $ml^{-1}$). Conditions as in Fig. 2.

centration of cefuroxim free human serum. The recoveries were calculated from the peak area ratio of cefuroxim. The recovery was 98–101% (Table 2). Actually the analytical recovery from the sample of cefuroxim in CE was 100% because there was no sample treatment except centrifugation.

### 3.3. Linearity and sample analysis

For quantitative analysis by MECC, the correlation between the peak area and the sample concentrations in the range 0.6–700 μg $ml^{-1}$ was studied. The linear regression equations for cefuroxim was $y = 1553.5x - 15.4$ ($r = 0.991$). This linearity guaranteed the determination of 250 μg $ml^{-1}$ range of cefuroxim in serum with a detection limit of 0.28 μM at a signal to noise ratio of 3. Because of the limited assay time and the small analyte volume in CE, the concentration limits of detection, in the best case, are about the same as in LC. However, the decrease by many orders of magnitude in analyte volume results in extremely low mass detection limits [13,14]. In CE, a typical concentration limit of detection of 0.28 μM corresponds to a mass LOD of 8.4 fmol with 31 nl injection compared with a mass LOD of 1.2 pmol with 20 μl for injection in HPLC.

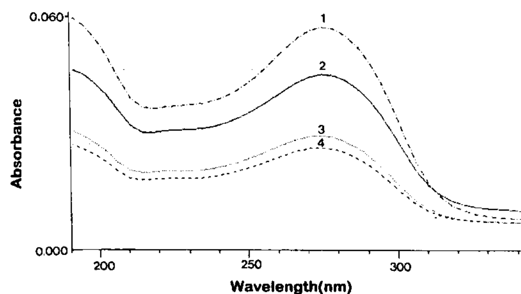


Fig. 4. UV spectrum of cefuroxim of the data in Fig. 2 compared with standard cefuroxim. 1, Standard in Fig. 2; 2, (A), 3, (B) and 4, (C) in Fig. 3.

The cefuroxim in patient's serum after injection (750 mg $ml^{-1}$) was separated by MECC. The samples were monitored at the time, 30, 60 and 90 min, respectively (Fig. 3). The cefuroxim concentration was found to be 52.9, 28.6 and 19.8 μg $ml^{-1}$ respectively. 19.8–52.9 μg $ml^{-1}$ were seen because of hemodilution at the beginning of extracorporeal circulation in patient showed much lower cefuroxim serum levels. Also, identification of cefuroxim was performed by characterizing the sample peak in terms of the migration time and UV spectrum (Fig. 4). The absorption spectra between 195 and 340 nm of the peak, which are assumed to be cefuroxim in Fig. 3 are compared with those of the standard drug. The excellent agreement between standard and sample indicates that MECC separation of these drugs in human serum is not subjected to interference by other components in the serum. Diode array scanning of the peak permits a quick and reliable confirma-

tion of the drugs. Desiderio et al. [15] have extensively investigated MECC determination of drugs in body fluids employing a diode array detector.

The CE method was developed for the analysis of cefuroxim which features direct injection of serum followed by MECC with diode array detection. Direct injection reduces sample preparation time and variability due to hydrolysis during the extraction procedure.

Compared to HPLC, the present method is superior at least in four respects: improved separation efficiency, simpler sample treatment, better selectivity and improved sensitivity. The number of theoretical plates obtained per meter was 182 000 for cefuroxim with the highest plate counts attainable by HPLC smaller than 2800. Separation of the standard by MECC corresponded to a mass LOD of 8.4 fmol, whereas HPLC separation obtained that of 1.2 pmol. Injection volumes in CE are less than about 31 nl compared to 20 μl for HPLC.

## 4. Conclusion

CE is applicable to analysis of cefuroxim in human serum of patients. CE shows an excellent run-to-run reproducibility and a good linearity in plot of concentration versus area under the curve. CE, in comparison with HPLC, has the potential to be used as a simple and economical analytical tool in the screening of cefuroxim in biofluids. The results described here show that the role of CE could be extended to quantitation and the monitoring of the drug in biofluids and clinical laboratories.

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