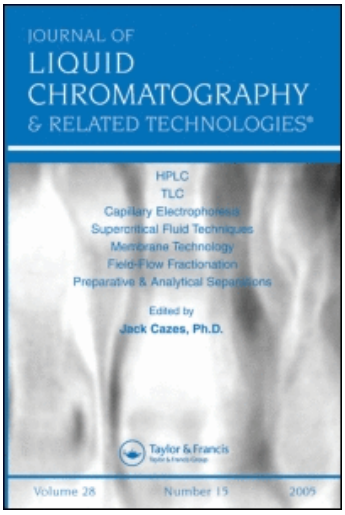

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SEPARATION OF CEFACLOR AND δ-3-CEFACLOR BY MICELLAR ELECTROKINETIC CHROMATOGRAPHY

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ABSTRACT

A new micellar electrokinetic chromatography method for the separation of cefaclor and δ-3-cefaclor using sodium cholate (SC) as an anionic surfactant, 35% (v/v) of acetonitrile as organic modifier and 3-cyclohexylamino-1-propane sulfonic acid (CAPS) as buffer electrolyte was developed. The influences of buffer pH, organic modifiers (including methanol and acetonitrile), and different surfactants (sodium cholate, sodium deoxycholate, and sodium dodecyl sulfate) on the separation of cefaclor and δ-3-cefaclor were investigated. Calibration line and reproducibility of the developed method were examined. The method was applied to determine active ingredients in cefaclor for oral dry suspension. The results were satisfactory.

INTRODUCTION

Cefaclor is widely used as antibiotic and antibacterial agents. It has been shown to be as effective as amoxicillin and cefazolin in the treatment of acute

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otitis media (1), acute maxillary sinusitis (2), and urinary tract infections.(3) It is also useful in the treatment of upper and lower respiratory tract infections and skin and skin structure infections.(4) δ-3-Cefaclor is one of the impurities in cefaclor formulation, which must be separated in the examination of cefaclor by liquid chromatography.(5) δ-3-Cefaclor and cefaclor are isomers. The difference in the isomers is the position of one double bond.

Currently, the methods for the determination of cefaclor are dominated by high performance liquid chromatography methods (HPLC).(6–10) For HPLC, the chromatographic column is not only expensive, but also easily contaminated and hard to clean. During the operation procedure, large amounts of organic solvent are needed. Column equilibration is time-consuming. Other methods for the determination of cefaclor include polarography (11), spectrofluorometer (12), and UV spectrophotometry.(13)

Capillary electrophoresis (CE) is a relatively recent separation technique with the advantages of high efficiency, small sample volumes, low solvent consumption, inexpensive column replacement, short analysis time, and the possibility of rapid method development.(14,15) Therefore, recently, there has been a noticeable increase in the use of CE for pharmaceutical analysis. The applications of this technique to the separation and/or determination of cephalosporins have previously been demonstrated using either micellar electrokinetic chromatography (MEKC) (16–23) or capillary zone electrophoresis.(18,24–27) But the application of CE to the separation of cefaclor and δ-3-cefaclor has not, hitherto, been reported.

In this paper, we report a MEKC method for the separation of cefaclor and δ-3-cefaclor, in which sodium cholate (SC) was used as a surfactant to form micelles, 35% (v/v) of acetonitrile was used as organic modifier, and 20 mmol/L of 3-cyclohexylamino-1-propane sulfonic acid (CAPS) was used as buffer electrolyte. The effects of organic modifiers (methanol and acetonitrile), pH of the background buffer, and the type of anionic surfactants on the separation of cefaclor and δ-3-cefaclor were investigated. The developed method was applied to determine active ingredients in cefaclor for oral dry suspension. The results were satisfactory.

EXPERIMENTAL

Instrumentation

A 270 A-HT capillary electrophoresis system (Applied Biosystems, Inc., USA), equipped with a UV detector was used in all the experiments. For data collection and data analysis, a N2000 software chromatography work station (purchased from ZheJiang University, China) was used. Polyimide-coated fused

silica capillaries with 72 cm total length and 50 μm internal diameter were obtained from YongNian Photoconductive Fiber Factory, Hebei, China. The detection window was located 22 cm from the end of the capillary. Pressure injection (5″ Hg, 2 s) was used. The UV detector was set at 264 nm for detection (maximum absorbance of cefaclor was obtained at 264 nm). The applied voltage was 20 kV. The capillary was thermostated at 30°C.

Reagents

Cefaclor and δ-3-cefaclor were obtained from National Institute for the Control of Pharmaceutical and Biological Products. Their chemical structures are illustrated in Figure 1. A 3.8 mg/mL of cefaclor stock solution was prepared by dissolving 19.0 mg of cefaclor in 5 mL of 0.27% (m/v) of sodium dihydrogen phosphate (pH 2.5). A 1.06 mg/mL of δ-3-cefaclor stock solution was prepared by dissolving 5.3 mg of δ-3-cefaclor in 5 mL of 0.27% (m/v) of sodium dihydrogen phosphate (pH 2.5). The solutions were stored at 4°C. Less concentrated standard solutions were prepared from the stock solutions by dilution, using distilled water as needed. A typical sample solution contained 0.2 mg/mL of cefaclor and 0.5 mg/mL of δ-3-cefaclor.

Sodium cholate (SC), sodium deoxycholate (SDC), β-cyclodextrin and 3-cyclohexylamino-1-propane sulfonic acid (CAPS) were purchased from Fluka. Sodium dodecyl sulfate (SDS) was obtained from Nacalail Tecque, Inc., Kyoto, Japan.

Unless otherwise specified, all chemicals were of analytical reagent grade. All solutions were prepared using filtered, degassed, and deionized distilled water.

Analytical Procedure

Prior to first use, a new capillary was first rinsed with deionized water for 10 minutes, followed by 1 mmol/L NaOH for 30 minutes, 0.1 mmol/L NaOH for

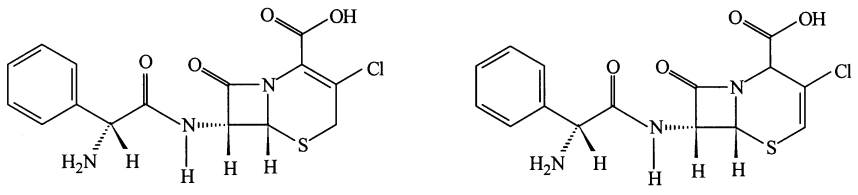

Figure 1. The structures of cefaclor and δ-3-cefaclor.

60 minutes, and then deionized water for 60 minutes. Between injections, the capillary was flushed with buffer for 10 minutes in order to optimize migration time and peak shape reproducibility.

Cefaclor for oral dry suspension was purchased from Lilly Suzhou Pharmaceutical Co., Ltd. Two bags of cefaclor sample were dissolved in 250 mL volumetric flasks using distilled water, then ultrasonacated for 10 minutes. A stock sample solution was prepared. The stock sample solution was diluted as needed using distilled water. The sample solutions were filtered using a 0.45 μm cellulose acetate syringe filter. The filtrates were then introduced directly into the CE system for the determination of cefaclor. After each run, the capillaries were purged by electrophoresis buffer for 10 minutes.

RESULTS AND DISCUSSION

Effects of Different Surfactants and pH of Background Buffer

In the paper, we studied the effects of three anionic surfactants on the separation of cefaclor and δ-3-cefaclor, including sodium cholate (SC), sodium deoxycholate (SDC), and sodium dodecyl sulfate (SDS). Figure 2 gives the separation electropherograms of the cefaclor isomers using different anionic surfactants as micelle-forming reagents. When SDS or SC, or SDC alone, was added in the background buffer containing 20 mmol/L of CAPS, cefaclor and δ-3-cefaclor were not separated completely. But when SC or SDC was used as a micelle-forming reagent, the peak shape of cefaclor and δ-3-cefaclor was better than that using SDS as a micelle-forming reagent.

The effect of pH of the background buffer containing 80 mmol/L of SDS, 20 mmol/L of CAPS, and 35% (v/v) of acetonitrile on the separation of cefaclor and δ-3-cefaclor was also investigated. The pH of these buffers was adjusted using concentrated sodium hydroxide solution. Figure 3 showed the separation electropherograms of cefaclor and δ-3-cefaclor at different pHs. In the pH range of 9.28–9.62, the cefaclor isomers can be separated at baseline. pH 9.35 was used in subsequent work.

Effect of Different Concentrations of Methanol and Different Surfactants

In order to improve the separation of cefaclor and δ-3-cefaclor, the influences of methanol on the separation of cefaclor and δ-3-cefaclor were investigated using three different anionic surfactants (including SDS, SC, and SDC). The effect of the organic modifier on the separation is complicated since the

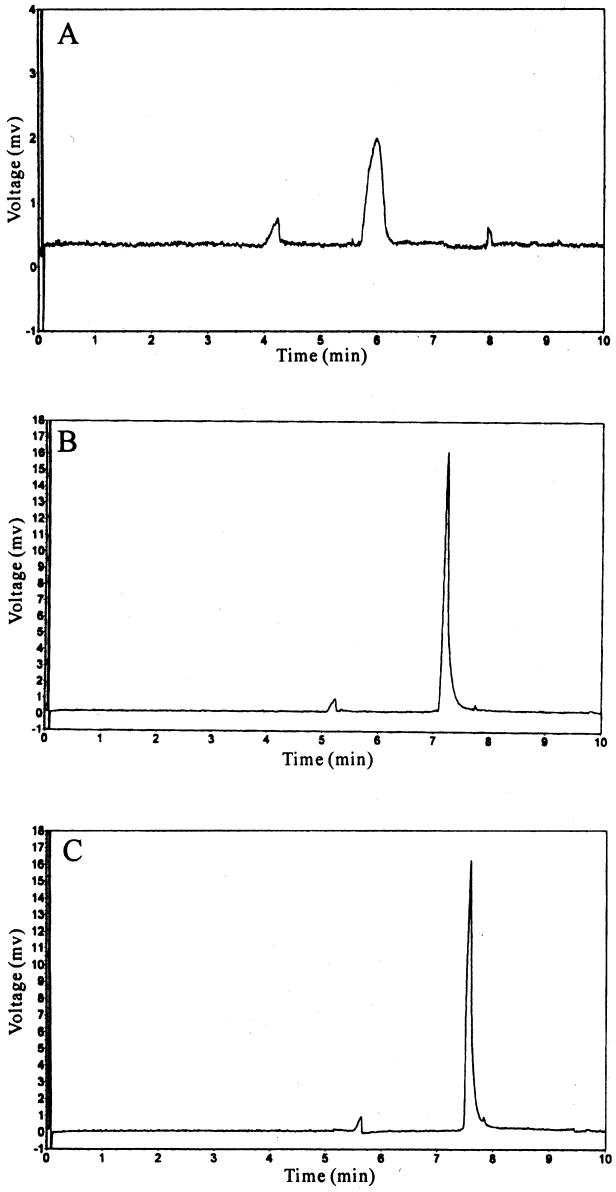

Figure 2. The separation electropherograms of the cefaclor isomers when SDS or SC, or SDC alone, was added in the background buffer containing 20 mmol/L of CAPS. Buffer: A, 80 mmol/L SDS + 20 mmol/L CAPS (pH = 9.69). B, 60 mmol/L SC + 20 mmol/L CAPS (pH = 9.26). C, 60 mmol/L SDC + 20 mmol/L CAPS (pH = 9.23).

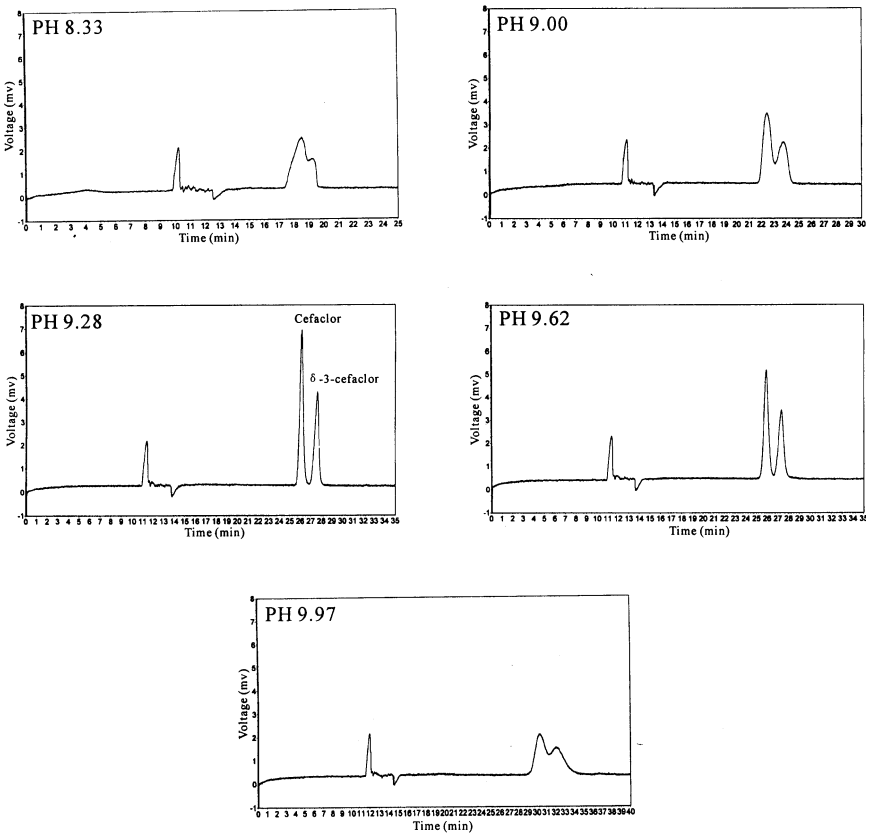

Figure 3. Effect of pH of the background buffer containing 80 mmol/L of SDS, 20 mmol/L of CAPS, and 35% (v/v) of acetonitrile on the separation of the cefaclor isomers.

organic modifier affects many parameters, e.g., the electro-osmotic velocity, critical micelle concentration (CMC), distribution coefficient, etc. Therefore, the kind and the concentration of organic modifiers must be optimized experimentally.

Firstly, when SDS was used as a micelle-forming reagent, the influence of different concentrations of methanol on the separation of cefaclor and δ-3-cefaclor was investigated. When the concentration of methanol was lower than 20% (v/v), the cefaclor isomers appeared in one peak. Figure 4A and 4B shows the separation electropherograms at 30% and 40% (v/v) of methanol. From Figure 4A and 4B, we can see that the cefaclor isomers were separated partially at 30% (v/v) of methanol and separated baseline at 40% (v/v) of

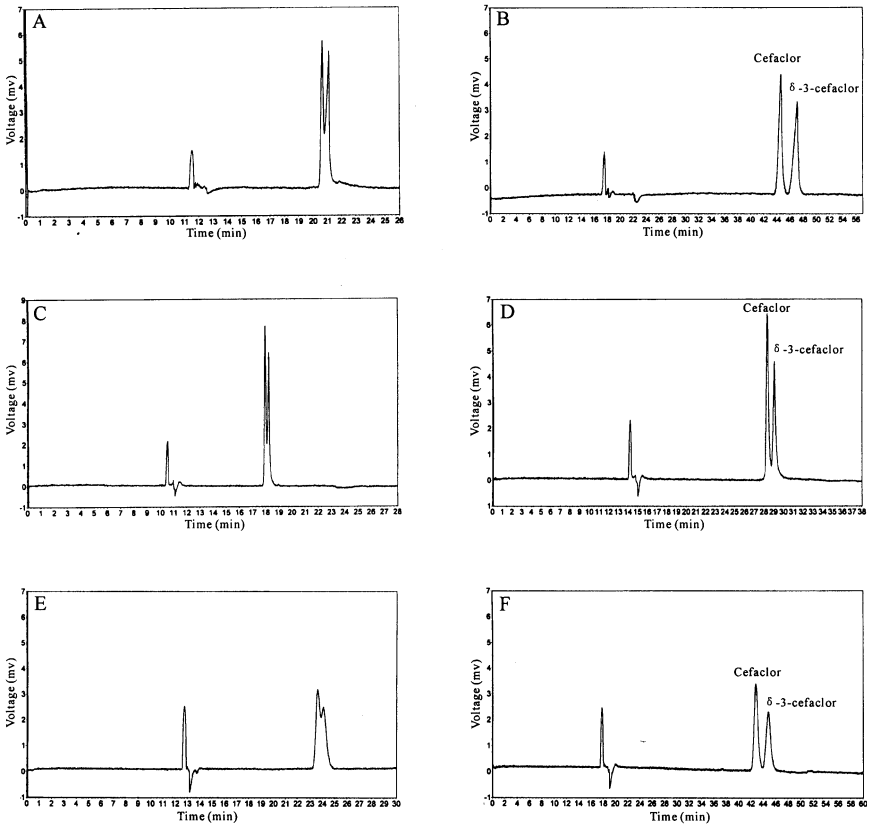

Figure 4. Effect of different concentrations of methanol using three different anionic surfactants on the separation of the cefaclor isomers. Buffer: A, 80 mmol/L SDS + 20 mmol/L CAPS + 30% (v/v) methanol. B, 80 mmol/L SDS + 20 mmol/L CAPS + 40% (v/v) methanol. C, 60 mmol/L SC + 20 mmol/L CAPS + 20% (v/v) methanol. D, 60 mmol/L SC + 20 mmol/L CAPS + 30% (v/v) methanol. E, 60 mmol/L SDC + 20 mmol/L CAPS + 30% (v/v) methanol. F, 60 mmol/L SDC + 20 mmol/L CAPS + 40% (v/v) methanol.

methanol. At too high a methanol concentration, the cefaclor isomers did not appear before 60 minutes, perhaps because SDS micelle formation was interfered.

Secondly, a similar work was performed when SC was used as a micelle-forming reagent. When the concentration of methanol was 10% (v/v), the cefaclor isomers were not separated completely. Figure 4C and 4D show the separation electropherograms at 20% and 30% (v/v) of methanol. From Figure 4C and 4D, we can see that the cefaclor isomers were separated partially at

20% (v/v) of methanol and nearly baseline separated at 30% (v/v) of methanol. When the concentration of methanol was 40% (v/v), the cefaclor isomers did not appear before 60 minutes.

Thirdly, a similar work was also done when SDC was used as a micelle-forming reagent. When the concentration of methanol was lower than 20% (v/v), the cefaclor isomers appeared in one peak. The separation electropherograms of cefaclor isomers at 30% and 40% (v/v) of methanol are illustrated in Figure 4E and 4F. Figure 4E and 4F show that the cefaclor isomers were separated partially at 30% (v/v) of methanol and baseline separated at 40% (v/v) of methanol.

Figure 5 shows the relationship of the migration time of cefaclor and the concentration of methanol using three different surfactants. From Figure 5, we can see that the migration time of cefaclor increased with the increasing of the concentration of methanol due to the decrease of electroosmotic velocity. Likewise, the migration time of δ-3-cefaclor also increased with the increasing of the concentration of methanol. The relationship of the resolution of cefaclor

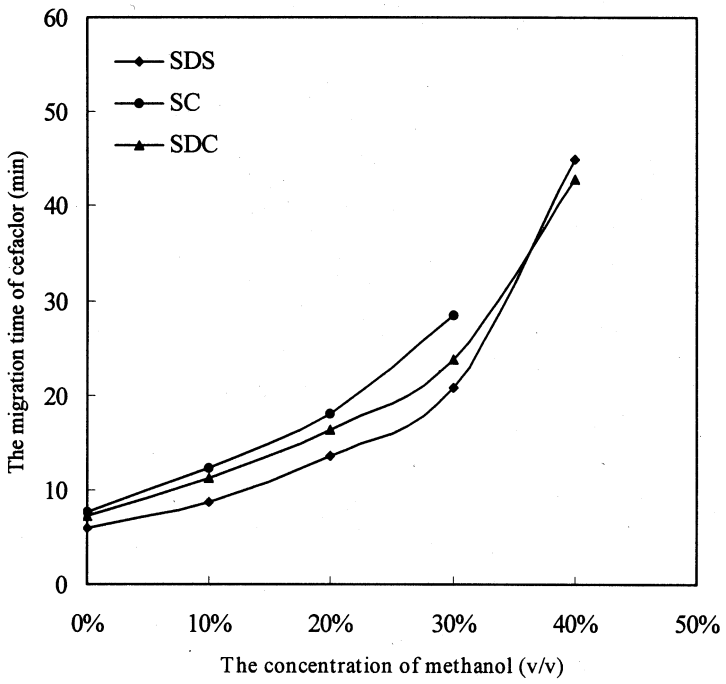

Figure 5. The relationship of the migration time of cefaclor and the concentration of methanol using three different surfactants.

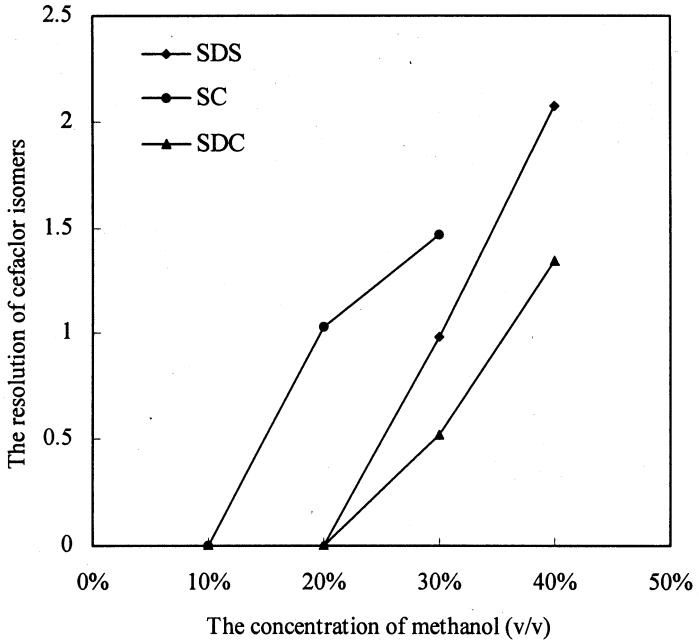

Figure 6. The relationship of the resolution of the cefaclor isomers and the concentration of methanol using three different anionic surfactants.

isomers and the concentration of methanol using three different anionic surfactants is illustrated in Figure 6. Figure 6 shows that the resolution of cefaclor isomers increased with the increasing of the concentration of methanol. From Figure 5 and Figure 6, we can see that at the same concentration of methanol, the migration time of cefaclor is longest and the resolution of cefaclor isomers is best when using SC as a micelle-forming reagent.

Effect of Different Concentrations of Acetonitrile and Different Surfactants

When methanol was used as the organic modifier, the migration times of cefaclor isomers were longer, although the cefaclor isomers can be separated baseline. Considering that the addition of acetonitrile led to a slight reduction of the BOF velocity, in order to shorten analysis time, the influence of different

concentrations of acetonitrile on the separation of cefaclor and δ-3-cefaclor was studied using three different anionic surfactants.

Firstly, when SDS was used as a micelle-forming reagent, the effect of different concentrations of acetonitrile on the separation of the cefaclor isomers was examined. When the concentration of acetonitrile was lower than 20% (v/v), the cefaclor isomers were not separated completely. At 30% (v/v) of acetonitrile, the cefaclor isomers were partially separated. When the concentration of acetonitrile was between 35% and 40% (v/v), the cefaclor isomers were separated baseline. Figure 7A shows the separation electropherogram of cefaclor isomers at 35% (v/v) of acetonitrile.

Secondly, a similar work was performed when SC was used as a micelle-forming reagent. When the concentration of acetonitrile was in the range of 10% to 20% (v/v), the cefaclor isomers were partially separated. When the concentration of acetonitrile was in the range of 30% to 40% (v/v), the cefaclor isomers were separated completely. Figure 7B shows the separation electropherogram of cefaclor isomers at 35% of acetonitrile.

Thirdly, a similar work was also done when SDC was used as a micelle-forming reagent. The cefaclor isomers were not separated completely at 10% (v/v) of acetonitrile. When the concentration of acetonitrile was in the range of 20% to 30% (v/v), the cefaclor isomers were partially separated. When the concentration of acetonitrile was in the range of 35% to 40% (v/v), the cefaclor isomers were baseline separated. Figure 7C shows the separation electropherogram of cefaclor isomers at 35% of acetonitrile.

The experimental results showed that when SC or SDC was used as a micelle-forming reagent, the peak shape of cefaclor isomers was sharper than that when SDS was used as a micelle-forming reagent. The relationship of the migration time of cefaclor and the concentration of acetonitrile using three different anionic surfactants is illustrated in Figure 8. From Figure 8, we can see that the migration time of cefaclor increased with the increasing of concentration of acetonitrile. With the increasing of concentration of acetonitrile, the migration time of cefaclor using SDS as a micelle-forming reagent increased more than when using SC or SDC as a micelle-forming reagent. Therefore, when the concentration of acetonitrile was from 30% to 40% (v/v), the separation of cefaclor and δ-3-cefaclor is faster using SC or SDC as a micelle-forming reagent than that using SDS as a micelle-forming reagent. Figure 9 shows the relationship of the resolution of cefaclor isomers and the concentration of acetonitrile using three different anionic surfactants. From Figure 9, we can see that the resolution of cefaclor isomers increased with the increasing of concentration of acetonitrile.

Compared with methanol, the separation time of cefaclor and δ-3-cefaclor was shortened when acetonitrile was used as the organic modifier. This is because the addition of acetonitrile leads to a slight reduction of the EOF velocity.

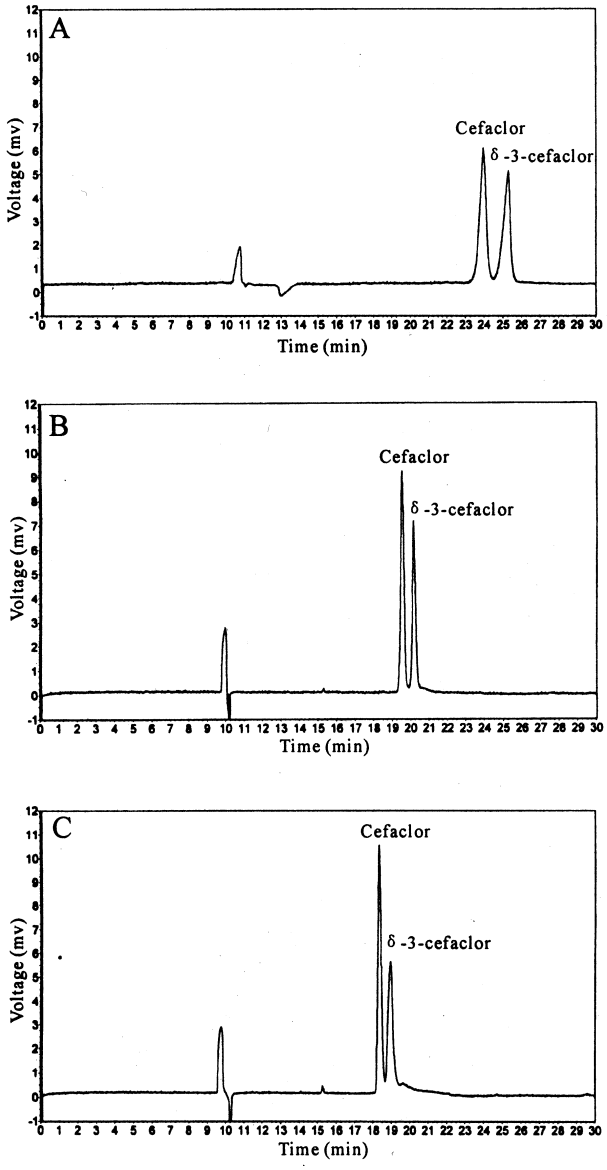

Figure 7. The separation electropherograms of the cefaclor isomers at 35% (v/v) of acetonitrile using three different anionic surfactants. Buffer: A, 80 mmol/L SDS + 20 mmol/L CAPS + 35% (v/v) acetonitrile. B, 60 mmol/L SC + 20 mmol/L CAPS + 35% (v/v) acetonitrile. C, 60 mmol/L SDC + 20 mmol/L CAPS + 35% (v/v) acetonitrile.

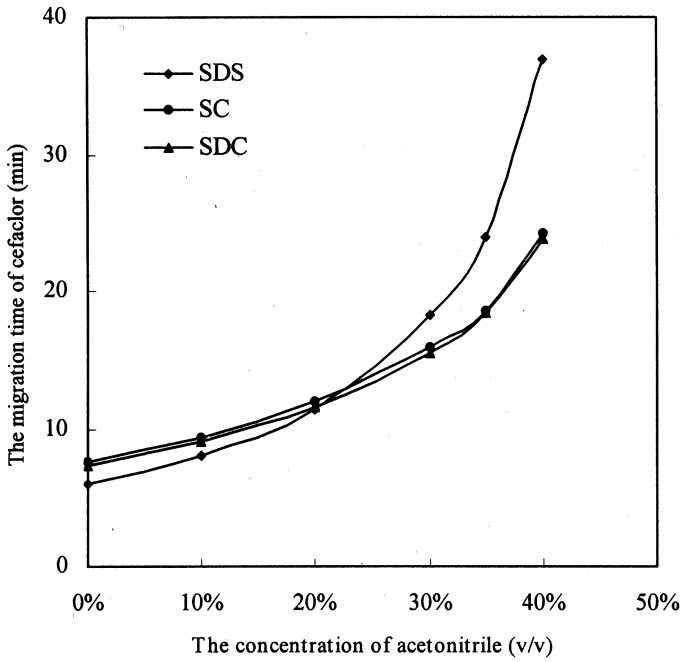

Figure 8. The relationship of the migration time of cefaclor and the concentration of acetonitrile using three different surfactants.

Quantitation

The correlation regression analysis was done on data obtained from different concentration levels of standard cefaclor and δ-3-cefaclor, while the buffer composition was 20 mmol/L of CAPS, 60 mmol/L of SC, 35% of acetonitrile (pH 9.33). The linear calibration ranges of cefaclor and δ-3-cefaclor are 0.01–2.0 mg/mL and 0.02–1.0 mg/mL, respectively. The regression equations are as follows:

$$\text{Cefaclor,} \quad A = 418360C + 10198 \ (R^2 = 0.9979)$$

$$\delta\text{-3-cefaclor,} \quad A = 138890C - 3983 \ (R^2 = 0.9989)$$

where A is the peak area in mAU · sec and C is the concentration of each analyte in mg/mL.

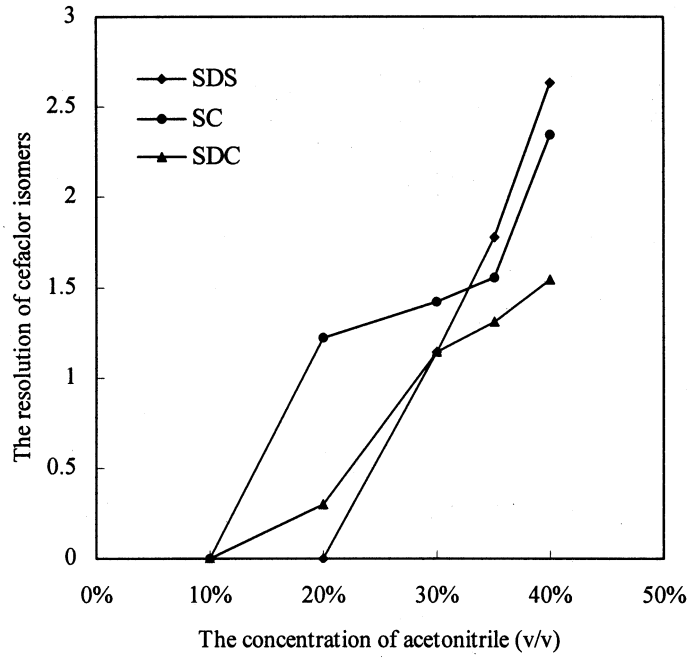

Figure 9. The relationship of the resolution of the cefaclor isomers and the concentration of methanol using three different anionic surfactants.

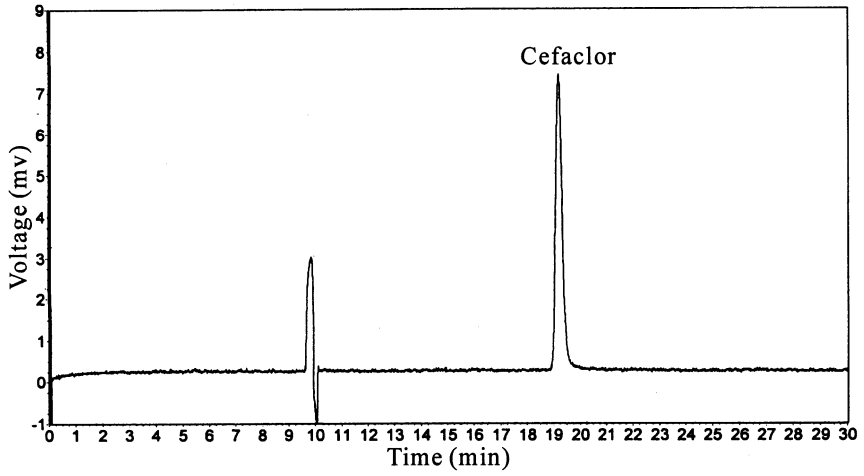

Figure 10. The electropherograms of the cefaclor sample. The conditions are shown in the text.

Table 1. Analysis Results of Cefaclor Sample

Sample	Determined Content[a] (mg)	RSD[b] (%)	Standard Content (mg)	Relative Deviation (%)	Added (mg/ml)	Recovery[c] (%)
Cefaclor for oral dry suspension	123.1	0.84	125	1.5	0.2	100.6 ± 4

[a]Average of three determinations.
[b]Abbreviation of relative standard deviation.
[c]Mean ± relative standard deviation (n = 3).

Sample Analysis

The cefaclor samples were analyzed using the MEKC method developed here. Cefaclor in dry suspension samples was identified by comparing its migration time with that of the standard. Pure standard was also added to samples so that the peak area of cefaclor was increased. In the cefaclor sample, δ-3-cefaclor was not detected. Figure 10 shows the electropherograms of the cefaclor sample. The sample analysis results are shown in Table 1. These results showed that this MEKC method was suitable for the determination of cefaclor in cefaclor for oral dry suspension.

CONCLUSIONS

In this paper we developed an MEKC method for the separation of cefaclor and δ-3-cefaclor. When SDS or SC or SDC was added, alone, in the buffer containing 20 mmol/L of CAPS, the cefaclors were not separated completely. When methanol or acetonitrile was used as an organic modifier, the cefaclor isomers can be baseline separated. Compared with methanol, the separation time of cefaclor and δ-3-cefaclor was shortened when acetonitrile was used as an organic modifier. When acetonitrile was used as the organic modifier, the peak shape of cefaclor isomers using SC or SDC as a micelle-forming reagent was sharper than that using SDS as a micelle-forming reagent. The developed method was applied to determine active ingredients in cefaclor for oral dry suspension successfully.

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REFERENCES

1. Solís, G.; Ochoa, C.; Pérez Méndez, C. Intl. J. Ped. Otorhinolaryngol. **2000**, *56* (3), 175.
2. Bandak, S.; Bolzon, L.; Johns, D.; Henle, S.; Allen, B.C. Therapeut. Res. **1999**, *60* (4), 185.
3. Leigh, A.P.; Nemeth, M.A.; Keyserling, C.H.; Hotary, L.H.; Tack, K.J. Clin. Therapeut. **2000**, *22* (7), 818.
4. Meyers, B.R. Clin. Therapeut. **2000**, *22* (2), 154.
5. European Pharmacopoeia-Supplement **1999**, 349.

6. Pehourcq, F.; Jarry, C. J. Chromatogr. **1998**, *812*, 159.
7. Moore, C.M.; Sato, K.; Katsumato, Y. J. Chromatogr. **1991**, *539*, 215.
8. Hsu, M.C.; Lin, Y.S.; Chung, C.H. J. Chromatogr. **1994**, *692*, 67.
9. Patel, Y.P.; Shah, N.; Bhoir, I.C.; Sundaresan, M. J. Chromatogr. **1998**, *828* (1–2), 287.
10. Gupta, S.; Prasad, B.B. J. Pharm. Biomed. Anal. **2000**, *23* (2–3), 307.
11. Rodrigues, L.N.C.; Zanoni, M.V.B.; Fogg, A.G. J. Pharm. Biomed. Anal. **1999**, *21* (3), 497.
12. Hefnawy, M.; El-Shabrawy, Y.; Belal, F. J. Pharm. Biomed. Anal. **1999**, *21* (4), 703.
13. Yi, Y.L.; Chin. J. Antibiot. **2000**, *25* (2), 153.
14. Jandik, P.; Bonn, G. *Capillary Electrophoresis of Small Molecules and Ions*; VCH: Weinheim, 1993.
15. Kuhn, R.; Hoffstetter-Kuhn, S. *Capillary Electrophoresis: Principles and Practice*; Springer: Heidelberg, 1993.
16. Mrestani, Y.; Neubert, R. J. Chromatogr. **2000**, *871* (1–2), 439.
17. Choi, O.K.; Song, Y.S. J. Pharm. Biomed. Anal. **1997**, *15*, 1265.
18. Castaneda Penalvo, G.; Kelly, M.; Maillois, H.; Fabre, H. Anal. Chem. **1997**, *69*, 1364.
19. Castaneda Penalvo, G.; Julien, E.; Fabre, H. Chromatographia **1996**, *42*, 159.
20. Chen, S.H.; Wu, H.L.; Wu, S.M.; Kou, H.S.; Lin, S.J. J. Chin. Chem. Soc. **1996**, *43*, 393.
21. Sciacchitano, C.J.; Mopper, B.; Specchio, J.J. J. Chromatogr. **1994**, *657*, 395.
22. Nishi, H.; Tsumagari, N.; Tsumagari, T.; Kakimoto, S.; Terabe, S. J. Chromatogr. **1989**, *477*, 259.
23. Nishi, H.; Tsumagari, N.; Tsumagari, T.; Kakimoto, S.; Terabe, S. Anal. Chem. **1989**, *61*, 2434.
24. Lin, C.E.; Chen, H.W.; Lin, E.C.; Lin, K.S.; Huang, H.C. J. Chromatogr. **2000**, *879* (2), 197.
25. Fabre, H.; Castaneda Penalvo, G. J. Liq. Chromatogr. **1995**, *18*, 3877.
26. Mrestani, Y.; Neubert, R.; Hartl, A.; Wohlrab, J. Anal. Chim. Acta **1997**, *349*, 207.
27. Mrestani, Y.; Neubert, R.; Schiewe, I.; Hartl, A. J. Chromatogr. **1997**, *690*, 321.

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