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Short communication

# Chiral separation of cefadroxil by capillary electrochromatography

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**Abstract**

In this paper, the chiral separation of cefadroxil was studied by capillary electrochromatography. Monolithic capillary column was prepared for the separation of cefadroxil enantiomers. The optimum buffer contained 28.5 mmol/L sodium acetate, 0.95% (v/v) acetic acid, 19 mmol/L β-cyclodextrin (β-CD) and 5% (v/v) isopropanol in formamide solution (pH 7.0), with the running voltage of 12 kV, the UV detector wavelength of 254 nm, the sample injected time of 8 s and the temperature of 25 °C. Under these conditions, the column efficiency of cefadroxil enantiomers were $N_1 = 5324$ and $N_2 = 23{,}768$ with a selectivity factor ($\alpha$) of 1.056 and resolution ($R_s$) of 0.978. The effect of buffer pH value, β-CD concentration, organic modifier (isopropanol) concentration and voltage was also investigated for the separation by CEC.


*Keywords:* Capillary electrochromatography; Cefadroxil; Chiral separation

## 1. Introduction

Many chiral pharmaceutical preparations are commonly administered as racemic mixtures. Usually both enantiomers do not possess the same physicological properties and potency and results in diverse absorption, metabolism and elimination rates. Furthermore, in some cases the non-active enantiomers can be responsible for side effects of different degree toxicology [1]. Consequently, the availability of analytical separation method with high efficiency and separation capability is very important in studying and understanding the pharmacological activity and pharmacokinetics of chiral drugs. Chromatographic methods typically employed for chiral separations include high-performance liquid chromatography (HPLC), gas chromatography (GC) and capillary electrophoresis (CE).

In the field of chiral separation, capillary electrophoresis (CE) is recognized as a challenging and powerful analytical tool allowing the use of very few quantities of expensive chiral selectors for enantiomeric separation [2–5]. Recently, capillary electrochromatography (CEC) using chiral-selector-coated capillaries, chiral mobile phase additives or packed the capillary with chiral stationary phase was successfully applied to enantiomers separation [6–13]. Capillary electrochromatography (CEC) combines the high-resolution capability and efficiency of CE with the high selectivity of HPLC. Analytes can be separated on the basis of the chromatographic principle of different partition and electrophoretic mobility. Lelivere et al. studied β-cyclodextrin and related derivatives bonded onto silica particles and resolution of a number of compounds was demonstrated [6]. Wolf et al. studied that chiral stationary phases based on Naproxen and Whelk-O materials were immobilized on 3 μm silica supports [7]. A weak anion-exchanger stationary phase was used by Lammerhofer and Lindner to separate amino acids by CEC [8]. Vancomycin, a macrocyclic antibiotic, had been applied to CEC and had been successful in the separation of a number of compounds of pharmaceutical interest [9]. Zheng et al. studied the separation of three acidic enantiomers (carprofen, coumachlor and warfarin) on a capillary column packed with 5 μm Whelk-O chiral stationary phase [10]. Schmid et al. studied the enantiomers separation of glycyldipeptides by CEC on a capillary packed with teicoplanin aglycone immobilized on 3.5 μm silica gel [11]. Kang et al. studied that a silica-bonded chiral-β-Dex chiral monolithic column by Sol-gel chemistry was used to separate neutral and acidity pharmaceutical [12]. Yang et al. introduced a silica-bonded bovine serum albumin chiral

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monolithic column for tryptophan enantiomeric separation by CEC [13].

Cefadroxil is a kind of antibiotic possessing good antibiotic action to Gram-positive bacterium. It has certain antibiotic activity to Gram-negative bacterium and part anaerobic bacteria and is clinically applied to the treatment of sore throat, tonsillitis, trecheitis, pneumonia and otitis media. So far there is still no report about the separation of chiral cefadroxil drug. In this paper, the separation of cefadroxil enantiomers was studied through CEC. Monolithic capillary column was prepared to separate cefadroxil enantiomers. The effect of pH value, β-CD concentration, organic modifier (isopropanol) concentration and voltage on the separation of cefadroxil enantiomers was investigated. Fine electropherogram of cefadroxil enantiomers separation was obtained under the optimum buffer condition. Cefadroxil was prepared through mixture acid anhydride method.

## 2. Experiment

### 2.1. Apparatus and materials

The experiment was performed on a 1229 HPCE analyzer (Beijing Institute of New Technology and Application, Beijing, China). The uncoated capillary (100 μm i.d., total length 60 cm and effective length 45 cm) came from Yongnian Optical Fiber Factory, Hebei Province, China. The pHS-3C meter came from Shanghai Leici Instrument Factory, Shanghai, China.

Isopropanol was from Beijing Chemical Factory (Beijing, China), β-cyclodextrin from Shanghai Xinxing Chemical Reagent Institute (Shanghai, China), cefadroxil from Shijiazhuang Pharmacy Group Co. Ltd. (Shijiazhuang, China, degree of purity >98%; using mixture acid anhydride method for preparation), *N*-methylformamide, 3-methacryloxypropyltrimethoxysilane (Bind-Silane) and bis-acrylamide from Sigma (USA). Vinylsufonic acid, lauryl acrylate and *N*,*N*,*N*′,*N*′-tetramethylenediamine (TEMED) was from Aldrich Chemical Company (USA), acrylamide from Linhai Chemical Company (China), poly(ethylene glycol) (PEG, MW 10,000) from Shanghai Chemical Reagent First Factory (Shanghai, China) and ammonium persulfate from Shenyang Xinxi Reagent Factory (Shenyang, China). The other chemical reagents used in the experiment were all of analytical grade and double-distilled water was used throughout.

### 2.2. Column preparation

Activation of capillary: fused silica capillary (100 μm i.d., total length 60 cm) was purged with 1 mol/L sodium hydroxide, distilled water, 1 mol/L hydrochloric acid, distilled water for 30 min respectively. A 50% (v/v) Bind-silane (bifunctional reagent) solution in acetone was then introduced into the capillary and left for 50 min. Finally, the capillary was rinsed with acetone and water for 15 min.

Polymerization procedure (refered to reference [14]): 30 mg of acrylamide, 60 mg of bis-acrylamide, 24.8 μL of vinylsulfonic acid, 60 mg poly(ethylene glycol) and 24.5 μL of lauryl acrylate were dissolved in 1.85 mL of *N*-methylformamide containing 100 μL of 100 mmol/L Tris–150 mmol/L boric acid (pH 8.2). During the polymerization step, 4 μL of 100% TEMED (*N*,*N*,*N*′,*N*′-tetramethylenediamine) and 10 μL of 40% ammonium persulfate were added to 0.5 mL of the above monomer solution. The polymerization proceeded overnight at room temperature under certain pressure. The capillary column was then washed for 3 h with an appropriate buffer, and purged with compressed nitrogen gas for another 3 h.

### 2.3. Procedures

Before the start of experimentation, the capillary was purged with methanol, water and running buffer respectively for 15, 15 and 10 min in sequence. Between the two runs, the capillary was only purged with buffer for 10 min. After three runs, the capillary was purged with the above procedure.

The running voltage was 12 kV, the UV detector wavelength 254 nm, the temperature 25 °C. The sample was injected by hydrodynamic mode for 8 s (12 cm high difference).

## 3. Results and discussions

The structure of cefadroxil is illustrated in the USP 28. Different electrophoresis modes were tested in the test. The effect of buffer pH value, β-CD concentration, organic modifier (isopropanol) concentration and voltage was also investigated for the separation of cefadroxil by CEC.

### 3.1. Selection of separation mode for cefadroxil chiral separation

Chiral separation of cefadroxil was investigated by capillary zone electrophoresis. Separation did not happen under 40 mmol/L phosphate buffer in water with different β-cyclodextrin (β-CD) concentration (from 10 to 40 mmol/L, 10 mmol/L interval. higher β-CD was dissolved in the buffer through using a little formamide and heating slightly. the same below). The affinity capillary electrophoresis was also introduced to study the chiral separation of cefadroxil, but the result was no better than the first one under the condition of 40 mmol/L phosphate buffer in water containing BSA (concentration from 20 to 120 μmol/L, 20 μmol/L interval). It turned out the same in nonaqueous capillary electrophoresis (the buffer contained 30 mmol/L sodium acetate, 1% (v/v) acetic acid in formamide solution and 10–40 mmol/L β-CD) (using uncoated capillary, 75 μm i.d., total length 60 cm and effective length 45 cm). Finally, capillary electrochromatography mode was used to study the chiral separation of cefadroxil. The effect of pH value, β-CD concentration, isopropanol concentration and voltage on the chiral separation was investigated in details.

### 3.2. Effect of β-CD concentration on cefadroxil chiral separation

The buffer consisted of 30 mmol/L sodium acetate, 1% (v/v) acetic acid in formamide solution and β-CD concentration vary-

ing from 10 to 40 mmol/L (10 mmol/L interval). The effect of β-CD concentration on the chiral separation of cefadroxil was investigated. It showed that changing β-CD concentration did not cause the complete separation of cefadroxil enantiomers, nor the noticeable change of the migration time of cefadroxil. When β-CD concentration was 20 and 30 mmol/L, the electropherogram top of cefadroxil enantiomers showed a little separation. In the case of other β-CD concentration, only one peak could be observed. So 20 mmol/L β-CD concentration was selected.

### 3.3. Effect of pH value on cefadroxil chiral separation

The buffer consisted of 30 mmol/L sodium acetate, 1% (v/v) acetic acid and 20 mmol/L β-CD in formamide solution (pH 7.0). The pH value of buffer was adjusted with 1 mol/L hydrochloric acid to 7.0, 6.8, 6.6 and 6.4, respectively. It showed that the chiral separation of cefadroxil was not found by changing pH values. The migration time of cefadroxil decreased as the pH value decreased. Because cefadroxil enantiomers had not been separated through varying pH value, the above solution without adding hydrochloric acid was the base of next study.

### 3.4. Effect of isopropanol concentration on cefadroxil chiral separation

The buffer consisted of 30 mmol/L sodium acetate, 1% (v/v) acetic acid, 20 mmol/L β-CD solution and isopropanol in formamide solution. The effect of different isopropanol concentration on the chiral separation of cefadroxil was investigated. The chiral separation of cefadroxil was achieved by changing isopropanol concentration from 2.5% to 30% (v/v). When isopropanol concentration was 2.5%, baseline chiral separation of cefadroxil was not achieved. When isopropanol concentration was 5%, baseline chiral separation of cefadroxil was achieved and the shapes of enantiomers peaks were good, which was shown in Fig. 1. When isopropanol concentration was higher than 5% (v/v), baseline separation of cefadroxil enantiomers was also achieved, but the migration time of the enantiomers would be too long. The negative peak in Fig. 1 was the EOF. It was caused mainly of electric double layer and showed the vacancy of formamide. Table 1 shows the relationship of isopropanol concentration with migration time, selectivity factor and resolution of the cefadroxil enantiomers (voltage: 12 kV).

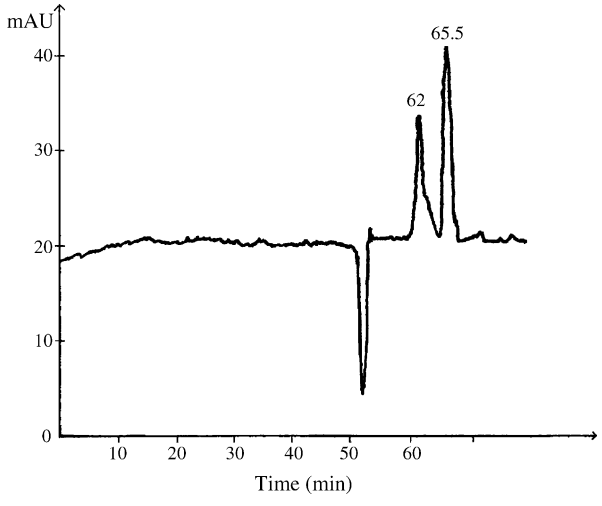


Fig. 1. Electropherogram of cefadroxil chiral separation. Experimental condition: monolithic capillary column; the buffer contained 28.5 mmol/L NaAc, 0.95% (v/v) HAc, 19 mmol/Lβ-CD and 5% (v/v) isopropanol in formamide solution (pH 7.0); applied voltage: 12 kV; UV wavelength: 254 nm; sample injected time: 8 s.

Table 1
The relationship of isopropanol concentration with migration time, selectivity factor and resolution of the cefadroxil enantiomers (12 kV)

| Isopropanol content (v/v) | $t_1$ (min) | $t_2$ (min) | $\alpha$ | $R_s$ |
|---|---|---|---|---|
| 2.5 | 58.3 | 61 | 1.046 | 0.675 |
| 5 | 62 | 65.5 | 1.056 | 0.978 |
| 8 | 71 | 76 | 1.070 | 1.091 |
| 10 | 74 | 79 | 1.071 | 1.480 |
| 15 | 83 | 88 | 1.065 | 1.507 |
| 20 | 91 | 97 | 1.064 | 1.463 |
| 30 | 127 | 132 | 1.039 | 1.515 |

So the optimum buffer consisted of 28.5 mmol/L sodium acetate, 0.95% (v/v) acetic acid, 19 mmol/L β-CD and 5% (v/v) isopropanol in formamide solution (pH 7.0). Thus it can be seen that isopropanol has an important effect on the separation of cefadroxil enantiomers. β-CD itself could cause the partial separation of cefadroxil enantiomers, while after mixed with isopropanol, β-CD had got obvious separation ability, which brought the chiral separation of cefadroxil. The separation mechanism could probably be based on synthetical result of hydrophobic interaction, wrapping up action and hydrogen bond. The base of cefadroxil chiral separation was the applying of monolithic capillary column, which could influence the electroosmotic mobility and wrapping up action. Possessing the characteristics of inner cavity hydrophobicity and external hydrophily, β-CD could not only form inclusion complexes with cefadroxil enantiomers but also improve their solubility and stability and thermodynamic properties. The effect of isopropanol showed in the following three aspects. First, it probably changed the stability of inclusion complexes and produced more stable ternary complexes of β-CD–cefadroxil–isopropanol; second, it possibly enlarged the difference of hydrogen bond action; third, it changed zeta potential and viscosity.

### 3.5. Effect of voltage on cefadroxil chiral separation

Under 28.5 mmol/L sodium acetate, 0.95% (v/v) acetic acid, 19 mmol/L β-CD and 5% (v/v) isopropanol in formamide solution (pH 7.0), The running voltage on the effect of cefadroxil chiral separation was tested. When the voltage changed from 12 to 16 kV, the migration time of cefadroxil decreased evidently. When voltage was 16 kV, the migration time of cefadroxil was 32 min. When voltage was lower than 14 kV, cefadroxil enantiomers could still be separated. When voltage was greater than 14 kV, the separation did not happen, with one peak being seen in the electropherogram. That could probably be explained in this way: the increasing voltage brought uprising Joule heat, varying solution viscosity and shortened migration time; meanwhile,

the effective mobility difference of cefadroxil enantiomers disappeared gradually. So 12 kV was selected.

Under the optimum condition, the column efficiency of cefadroxil enantiomers was $N_1 = 5324$ and $N_2 = 23{,}768$ with a selectivity factor ($\alpha$) of 1.056 and resolution ($R_s$) of 0.978.

### 3.6. Reproducibility

Reproducibility tests based on five sample injection were performed. The results showed that the reproducibilities of migration time and peak areas for the cefadroxil enantiomers were satisfactory. The R.S.D. values of migration times and peak areas were respectively below 3.2% and 5.1%.

## 4. Conclusion

The chiral drug cefadroxil was successfully separated by capillary electrochromatography. Nonaqueous solvent was used in the electrochromatography experiment. It demonstrated that capillary electrochromatography possessed superiority in chiral separation aspect.

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