Enantiomeric Separation of Amphetamine and Phenylephrine by Capillary Zone Electrophoresis with Hydroxypropyl-β-cyclodextrin as Chiral Additive

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1 Introduction

Capillary electrophoresis (CE) has been widely used for chiral separation together with gas chromatography (GC) and high performance liquid chromatography (HPLC) [1,2]. Several separation modes including capillary zone electrophoresis (CZE) [3–6], micellar electrokinetic chromatography (MECC) [7,8], capillary electrochromatography (CEC) [9] and capillary gel electrophoresis (CGE) [10] have been used for this purpose. Until now, CZE with cyclodextrins (CDs) as chiral selector has been most widely used and proved to be the most successful chiral CE systems.

Recently enantioseparation for nine amphetamine-related drugs has been reported by Cladrowa-Runge *et al.* [6] using CZE with native and various derived β-cyclodextrins, including HP-β CD, as chiral selector. However, they did not optimize the concentration of chiral selectors and the enantiomers of amphetamine were not well separated when using low concentration of HP-β-CD where the separation of phenylephrine was not mentioned.

In our previous work [5], we have successfully separated the enantiomers of amphetamine and phenylephrine by using CD-mediated CZE. Here, we present a further study on the enantiomer separation of the two drugs by using hydroxypropyl-β-cyclodextrin (HP-β-CD) as chiral selector. In this work, much better separation for amphetamine enantiomers was accomplished, compared with our previous result, and the enantiomer separation of phenylephrine was reached to a much higher degree, with resolution (R_S) being 4.2.

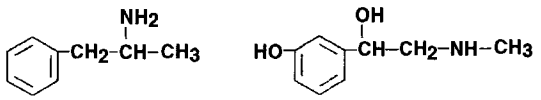
2 Experimental

2.1 Apparatus

Experiments were carried out on a laboratory-assembled CE system. An uncoated fused silica capillary of 62 cm length (effective length 41 cm) with 50 μm i.d. × 375 μm o.d. (Yongnian Optical Fiber Factory, Hebei, China) was used as a separation tube. A laboratory-made high-voltage power supply that can provide voltage up to 30 kV was used to drive the separation. On-column detection was performed at the cathode on a CV[4] UV detector (ISCO, Lincoln, NE, USA) at 210 nm with a rise time of 0.8 s. Electropherograms were recorded on an SE 120 recorder (ABB Goerz Instruments, Vienna, Austria). A small fan was used to dissipate the Joule heat generated. A pHs-3C pH meter with an E-201-C combination electrode (Rex Instrument Factory, Shanghai, China) was used for pH measurements.

2.2 Chemicals

HP-β-CD was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI 53233, USA). Tris(hydroxymethyl)aminomethane (Tris) was from Fluka (Buchs, Switzerland). Racemic amphetamine and phenylephrine (structure shown in **Figure 1**) were provided by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All other chemicals were of analytical grade. Doubly distilled water was used.



amphetamine **phenylephrine**

Figure 1. Molecular structures of amphetamine and phenylephrine.

2.3 Procedures

Tris-H_3PO_4 buffer (100 mM, pH 2.3) was prepared by dissolving 6.05 g of Tris in water, adjusting it to pH 2.3 with phosphoric acid, and diluting the solution with water to 500 mL in a volumetric flask. CDs were dissolved in the above buffer. Buffers were filtered through 0.45-μm membrane filters and degassed by sonication prior to use. The sample solution was prepared by dissolving the solute in Tris-H_3PO_4 buffer at an approximate concentration of 0.1 mg/mL so that adequate signals could be obtained.

The new capillary column was first vacuum rinsed with 1M NaOH and water for 30 min. each in order to activate the silica inner wall and then equilibrated with the operating buffer for 10 min. Between two consecutive injections, the capillary

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was rinsed with 0.1 M NaOH for 2 min, water for 2 min, and the operating buffer for 5 min. Samples were injected by the electrokinetic method at the anode and CZE operations were run under constant voltage at ambient temperature (about 14-17 °C).

The resolution between enantiomers was calculated by the equation:

$$R_S = 2 \times (t_2 - t_1) / (w_2 + w_1)$$

where t_2 and t_1 are the migration times (min) of the two enantiomers and w_2 and w_1 are the peak widths of each peak at the baseline (min).

3 Results and Discussion

In this study, the enantiomer separation of the two drugs was first investigated by using 50 mM Tris-H_3PO_4 buffer (pH 2.3) containing 12 mM of HP-β-CD. Near-baseline separation was achieved for the enantiomers of phenylephrine whereas amphetamine was only partially separated into its enantiomers in this case. To improve the enantiomer separation of the two drugs, the buffer concentration was optimized by changing the Tris-H_3PO_4 concentration between 50 and 200 mM. When the concentration of Tris-H_3PO_4 buffer was increased from 50 to 100 mM, the peak shape of amphetamine enantiomers became more symmetrical with smaller tailing and the resolution was improved, with R_S increasing from 0.54 to 0.88, whereas the enantioseparation for phenylephrine remained almost unchanged. On the other hand, the buffer concentration change in the range from 100 to 200 mM had little influence on the enantioseparation of the two drugs.

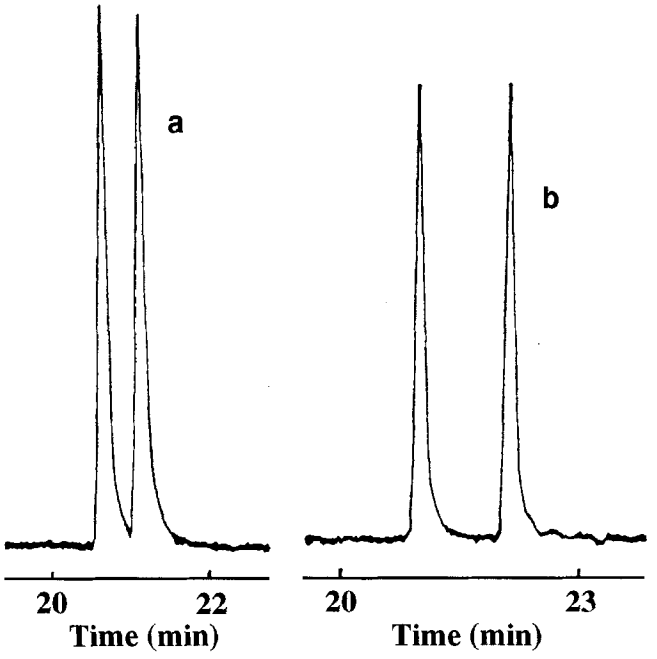

Figure 2. Electropherograms of the enantioseparation of amphetamine (a) and phenylephrine (b). Conditions, BGE, 100 mM Tris-H_3PO_4 (pH 2.3) containing 60 mM HP-β-CD; separation tube, 62 cm (41 cm to detector) × 50 μm i.d. × 375 μm o.d.; running voltage, 22 kV; detection 210 nm (0.005 AUFS); temperature ambient (about 15 °C).

Therefore, 100 mM Tris-H_3PO_4 buffer was chosen for the separation. For further improving the enantioseparation of the two drugs, the concentration of the chiral selector HP-β-CD was changed in the range from 3 to 60 mM. The best enantioseparation for both drugs was achieved at 60 mM HP-β-CD and the results are shown in **Figure 2**, where the complete enantioseparation was achieved for both drugs with the resolution for phenylephrine being much higher (R_S = 1.7 for amphetamine and 4.2 for phenylephrine).

Cladrowa-Runge *et al.* showed that when chiral selectors were all used at low concentration (10 mM), compared with native β-CD, HP-β-CD gave no advantages for the enantioseparation of amphetamine and some other amphetamine-related drugs [6]. Our work suggests that although HP-β-CD may give no advantages for the enantioseparation of some compounds compared with the native β-CD when both chiral selector were used at low concentrations, the enantioseparation of some compounds may be much improved by using higher concentration of HP-β-CD (it has a much higher solubility than native β-CD). Therefore the concentration optimization of chiral selector is necessary in chiral separations [11, 12].

4 Conclusion

The enantiomers of the drugs amphetamine and phenylephrine were investigated by CZE with HP-β-CD as chiral selector. The baseline enantiomer separation of the two drugs was achieved by using100 mM Tris H_3PO_4 (pH 2.3)–60 mM HP-β-CD buffer in CZE. The method can be easily operated with conventional reagents. The resolution achieved for the enantiomers of both drugs is sufficient for chiral quantitation.

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