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Short communication

Cyclodextrins as buffer additives for the enantiomeric separation of pinacidil in capillary zone electrophoresis

Zhi Wang[a], Aijin Huang[a], Yiliang Sun[a,*], Zengpei Sun[b]

[a]*Department of Chemistry, Peking University, Beijing 100817, P.R. China*

[b]*National Institute for the Control of Pharmaceutical and Biological Products, Beijing 100050, P.R. China*

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Abstract

The enantiomeric separation of pinacidil was studied using α-cyclodextrin (α-CD), β-CD, heptakis (2,6-di-O-methyl)-β-CD (DM-β-CD), hydroxypropyl-β-CD (HP-β-CD) and γ-CD as buffer additives at acidic pH in capillary zone electrophoresis. β-CD, HP-β-CD and γ-CD all showed chiral recognition on the compound, with HP-β-CD giving the highest chiral selectivity. Buffer concentration and HP-β-CD concentration were optimized for the separation. The separation was improved by the addition of hydroxypropyl cellulose (HPC) to the buffer. A complete separation of pinacidil enantiomers was achieved by using 100 m*M* Tris–H_3PO_4 buffer (pH 2.3) containing 9 m*M* HP-β-CD and 0.05% HPC.

Keywords: Buffer composition; Enantiomer separation; Pinacidil

1. Introduction

The enantiomer separation of optically active compounds continues to be an active area of research in gas chromatography (GC), liquid chromatography (LC) and more recently, capillary electrophoresis (CE), because in many cases pharmacological and toxicological effects of racemic drugs are related to only one of the enantiomers. Because of its high separation efficiency, relatively simple instrumental set-up, versatility in separation mode and low running cost, CE has gained rapid development in the past few years and has become a complementary analytical tool to GC and LC in chiral separation [1,2]. Until now, cyclodextrins (CDs) and their derivatives have been the most widely used and most successful chiral selectors in chiral CE [3–13].

Previous studies [3–13] have shown that various parameters, such as CD type and concentration, pH, composition and concentration of the running buffer, capillary temperature, capillary length, electric field strength and organic modifiers, can influence the chiral separation in CE.

The two enantiomers of the multiple-action antihypertensive drug pinacidil have been proven to present different pharmacological effects and HPLC has been used for the separation of pinacidil enantiomers [14].

In this paper, the enantiomeric separation of pinacidil was studied using capillary zone electrophoresis (CZE) with CDs as chiral selector. The separation conditions were optimized and a complete enantiomer separation of pinacidil was achieved.

*Corresponding author.

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This separation method can be operated conveniently and inexpensively by using only conventional reagents.

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 cm 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 from 0 to 30 KV was used to drive the separation. On-column detection was performed at 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 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

α-, β- and γ-CD were purchased from TCI (Japan). Heptakis(2,6-di-O-methyl)-β-CD (DM-β-CD) was obtained from Sigma (St. Louis, MO, USA). Hydroxypropyl-β-CD (HP-β-CD) and hydroxypropyl cellulose (HPC) were from Aldrich (Milwaukee, WI 53233, USA). Tris(hydroxymethyl) aminomethane (Tris) was from Fluka (Buchs, Switzerland). Racemic pinacidil (its structure is shown in Fig. 1) was kindly provided by National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All other chemicals were of analytical grade. Doubly distilled water was used.

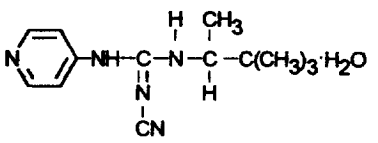

Fig. 1. Molecular structure of pinacidil.

2.3. Procedures

Tris–H_3PO_4 buffer (100 m*M*, pH 2.3) was prepared by dissolving 6.05 g of Tris in water, titrating 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 1 *M* NaOH and water for 30 min each in order to activate the silica on the wall and then equilibrated, with the operating buffer for 10 min. Between two consecutive injections, the capillary 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 an electrokinetic method at the anode and CZE operations were run under constant voltage at ambient temperature (about 14–17°C).

Resolution between enantiomers was evaluated 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

3.1. Effect of chiral selector

In this study, the enantiomer separation of pinacidil was first investigated by using 50 m*M* Tris–H_3PO_4 buffer (pH 2.3) containing 12 m*M* α-, β-, DM-β-, HP-β- and γ-CD respectively. When α-CD and DM-β-CD was used, no resolution was achieved for the pinacidil enantiomers; when β-CD, HP-β-CD and γ-CD were used, the enantiomers of pinacidil were partially separated to different degrees (see Fig. 2), with H-β-CD giving the best resolution (the resolution value, R_S, is 0.6). Therefore, the

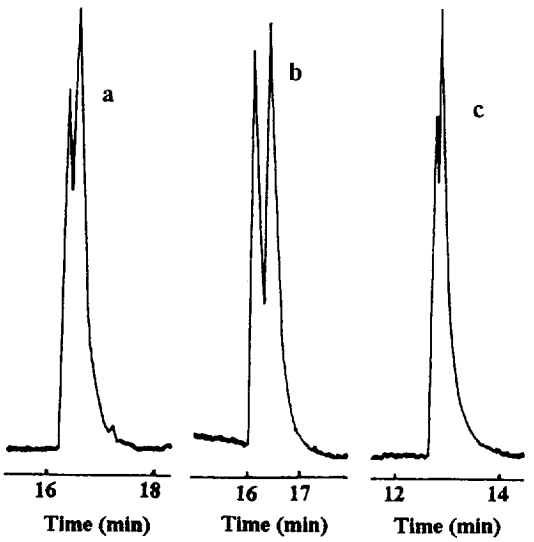

Fig. 2. Electropherograms of the enantioseparation of pinacidil. Conditions: background electrolyte (BGE), 50 m*M* Tris–H_3PO_4 (pH 2.3) containing 12 m*M* β-CD (a), 12 m*M* HP-β-CD (b) and 12 m*M* γ-CD (c); separation tube, 62 cm (41 cm to detector)×50 μm I.D.×375 μm O.D.; running voltage, 22 kV; detection, 210 nm (0.005 AUFS); temperature, ambient (about 15°C).

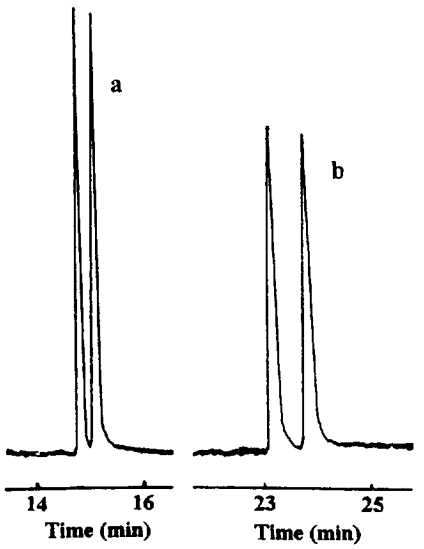

Fig. 3. Electropherograms of the enantioseparation of pinacidil. Conditions: (a) BGE, 100 m*M* Tris–H_3PO_4 (pH 2.3) containing 9 m*M* HP-β-CD; (b) BGE, 100 m*M* Tris–H_3PO_4 (pH 2.3) containing 9 m*M* HP-β-CD and 0.05% HPC. Other conditions as in Fig. 2.

chiral selector HP-β-CD was chosen for further studies of the enantiomeric separation of pinacidil.

3.2. Effect of buffer concentration

Rickard and Bopp [8] showed that buffer concentration has influences on chiral separation. In this study, the influence of buffer concentration on the enantiomer separation of pinacidil was investigated by changing the Tris–H_3PO_4 concentration from 50 to 100 m*M*. When the concentration of Tris–H_3PO_4 buffer was increased from 50 to 100 m*M*, the enantiomer separation of pinacidil was greatly improved (increased from 0.6 to 1.3) whereas the buffer concentration change in the range from 100 to 200 m*M* had little influence on the separation, with migration times being slightly increased with the increasing of buffer concentrations. Based on the above results, subsequent studies were carried out with the Tris–H_3PO_4 buffer concentration at 100 m*M*.

3.3. Effect of HP-β-CD concentration

The concentration of chiral selector is an important parameter in chiral separation by CE. At extremely low concentration, the amount of chiral selector is not sufficient to form complexes with enantiomers and therefore, the enantiomers can not be separated. On the other hand, when the concentration of chiral selector is extremely high, both enantiomers will be nearly completely complexed. Because the two enantiomer-chiral selector complexes have very similar mobilities, the enantiomers can not be separated either in this case. Wren and Row [10,11] proposed a theoretical model which relates the apparent mobility difference between the two enantiomers to the concentration of the chiral selector. The model suggests that the degree of separation depends on the concentration of chiral selector and there is an optimum concentration for chiral selector. Our experiment agrees with this prediction. The optimal HP-β-CD concentration for the enantiomeric separation of pinacidil was found at about 9 m*M*, where near-baseline separation (R_S 1.5) was achieved (Fig. 3a).

3.4. Effect of the addition of HPC to the buffer

The addition of small amount of hydrophilic polymer to the background electrolyte has been proven to improve some chiral separations in CE [12,13]. In this work, we added 0.05% HPC to 100 m*M* Tris–H_3PO_4 (pH 2.3)–9 m*M* HP-β-CD buffer for improving the separation. The result is shown in Fig. 3b. Compared with Fig. 3a, the enantiomeric separation of pinacidil was improved, with the R_S value being increased from 1.5 to 2.2. A complete separation of the enantiomers of pinacidil was achieved in this case.

4. Conclusion

The enantiomers of the drug pinacidil have been successfully separated by using CZE with CDs as chiral selector at acidic buffer (pH 2.3) with a small amount of HPC being added. The experiment shows that CD type and concentration, buffer concentration and the addition of HPC to the running buffer influence the separation. This separation method is simple, easy to operate and only involves the use of conventional reagents.

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References

[1] M. Novotny, H. Soni and M. Stefansson, Anal. Chem., 66 (1994) 646A.
[2] S. Terabe, K. Otsuka and H. Nishi, J. Chromatogr. A, 666 (1994) 295.
[3] S. Fanali, J. Chromatogr., 545 (1991) 437.
[4] K.D. Altria, D.M. Goodall and M.M. Rogan, Chromatographia, 34 (1992) 14.
[5] H. Nishi, Y. Kokusenya, T. Miyarnoto and T. Sato, J. Chromatogr. A, 659 (1994) 449.
[6] L.A. St. Pierre and K.B. Sentell, J. Chromatogr. B, 657 (1994) 291.
[7] M.W.F. Nielen, Anal. Chem., 65 (1993) 885.
[8] E.C. Rickard and R.J. Bopp, J. Chromatogr. A, 680 (1994) 609.
[9] Z. Wang, Y. Sun and Z. Sun, J. Chromatogr. A, 735 (1996) 295.
[10] S.A.C. Wren and R.C. Rowe, J. Chromatogr., 603 (1992) 235.
[11] S.A.C. Wren and R.C. Rowe, J. Chromatogr., 609 (1992) 363.
[12] J. Snopek, H. Soini, M. Novotny, E.S. Keulemansova and I. Jelinek, J. Chromatogr., 559 (1991) 215.
[13] D. Belder and G. Schomburg, HRC, 15 (1992) 687.
[14] K. Sakamoto and Y. Nakamura, Xenobiotica, 24 (1994) 329.