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Enantiomeric Separation of *d-/l*-Norepinephrine and -Epinephrine by High-Performance Liquid Chromatography with a β-Cyclodextrin Type Chiral Stationary Phase

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Enantiomeric separation of *d-/l*-norepinephrine (NE) and *d-/l*-epinephrine (E) was investigated with various mobile phases by high-performance liquid chromatography using a β-cyclodextrin type chiral stationary phase as a chiral column. The mobile phase of 2.5 M phosphate buffer (pH 3.0) gave separation factors (α s) of 1.04 and 1.06 for NE and E, respectively. © 1998 John Wiley & Sons, Ltd.

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INTRODUCTION

Norepinephrine (NE) and epinephrine (E) are neurotransmitters in mammals, which exhibit vasoconstriction and blood pressure elevation. Norepinephrine is produced from dopamine (DA) by DA β-hydroxylase (EC.1.14.17.1) and E from NE by *N*-methyltransferase (EC.2.1.1), both of which are *l*-forms having potent biological activities mentioned above.

Therefore, *l*-forms are usually used in pharmaceutical preparations such as *l*-E in the local anesthetic or ophthalmic solution. During long storage, however, racemization at the β-position from the amino group occurs. The activity of *d*-epinephrine is one tenth of that of *l*-form, hence, the optical purity of E in the preparation is important for adequate usage (Allgire *et al.* 1985; Peterson *et al.*, 1992). The optical rotation method was often adopted for this purpose; however, it is sometimes not applicable on account of low sensitivity (Allgire *et al.* 1985).

In recent years, high-performance liquid chromatography (HPLC) has been a useful tool for determination of optical purity of chiral compounds, employing a chiral stationary phase, a chiral additive in the mobile phase or a chiral derivatization reagent (Gorog *et al.*, 1994). Although the enantiomeric separation by HPLC of both NE and E were already reported after derivatization with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) and 2,3,4-tri-*O*-acetyl-α-D-arabinopyranosyl isothiocyanate (AITC) (Nimura *et al.*, 1981), the peaks of degradation and by-products derived from GITC and AITC appear to disturb the detection of the derivatives in the chromatogram. For the direct enantiomeric separation of catecholamines (CAs), a chiral ligand-exchange chromatography utilizing copper (II) chelate complexation was also adopted (Yamazaki *et al.*, 1991; Oi *et al.*, 1994). However, NE enantiomers were separated, whereas E enantiomers were not.

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On the other hand, capillary electrophoresis (CE) using the derivatized β-cyclodextrins (β-CD) as chiral selectors was reported to be effective for enantiomeric separation of CAs and applied to the determination of optical purity of *l*-epinephrine pharmaceuticals (Fanali, 1989; Peterson *et al.*, 1992). As a result, 2.2–2.3% of *d*-E were detected in pharmaceuticals which had expired by 12 months (Peterson *et al.*, 1992). Since HPLC is more widely used than CE, owing to its superior reproducibility and ease of operation, we aim to separate E and NE enantiomers directly by HPLC using a β-CD type chiral stationary phase, and apply the method to the purity examination of a E pharmaceutical.

EXPERIMENTAL

Chemicals. DL-Adrenaline(*d-/l*-epinephrine), L-noradrenaline bitartrate (*l*-norepinephrine) and L-adrenaline (*l*-epinephrine) were purchased from Tokyo Kasei. Co., Ltd (Tokyo, Japan). (±)-Norepinephrine L-bitartrate hydrate (*d-l*-norepinephrine) was from Wako Pure Chemicals (Osaka, Japan). Dopamine hydrochloride was from Sigma Co., Ltd. (St. Louis, MO, USA). Ultron ES-CD and Ultron ES-phCD columns (150 × 4.6 mm i.d.) were kindly donated by Sinwa Chemical Industry (Kyoto, Japan). Ammonium dihydrogenphosphate [$(NH_4)H_2PO_4$] and phosphoric acid were from Kanto Chemical Co., Ltd (Tokyo, Japan). Water was used after purification by Milli Q system (Nihon Millipore, Tokyo, Japan).

HPLC conditions. HPLC equipment consisted of an L-7100 intelligent pump, an L-4200 UV detector and a D-2500 integrator (Hitachi, Tokyo, Japan). Dual Ultron ES-CD columns were connected in series after a sample injector 7125 (Rheodyne, CA, USA) with 50 μL of loop. The column temperature was ambient, and UV absorption was detected at 260 nm. Mobile phases were 0.1, 0.5, 1.0, 1.5, 2.0 and 2.5 M (NH_4) H_2PO_4–H_3PO_4 buffer solutions (pH 3.0), and the flow rate was 0.5 ml/min.

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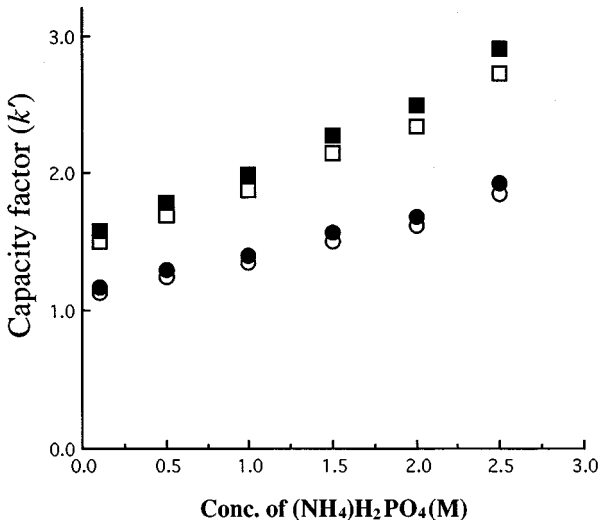

Figure 1. Effect of $(NH_4)H_2PO_4$ concentration in the mobile phase on capacity factors (*k*'s) of CAs. (■ *d*-E: □*l*-E; ●*d*-NE; ○*l*-NE).

Preparation of CA standard solutions. One millimolar of *dl*- or *l*-E, *dl*- or *l*-NE and DA were prepared in 1.0 M $(NH_4)H_2PO_4$–H_3PO_4 buffer solution (pH 3.0). Five microlitres of the solution for *l*-E, -NE and DA, and 10 μL of the solution for *dl*-E and -NE were injected in the column.

Application to pharmaceuticals. An injection including 1 mg/ml *l*-E (Bosmin, Daiichi Pharmaceutal Co., Ltd) was filtered with a Columnguard LCD_4 (Nihon Millipore, Japan) and 2 μL of the filtrate was injected into the column.

RESULTS AND DISCUSSION

Since CAs are unstable in pH above 6.0, the mobile phases above pH 6.0 could not be used for the separation of CAs. On account of a tolerance of the column, a buffer solution above pH 3.0 had to be used. Firstly, the mobile phases containing a phosphate buffer solution adjusted to pH 3.0, 4.0 and 5.0 were tested for the direct separation of CA enantiomers using a β-CD type chiral column (Ultron ES-CD). As a result, as pH 3.0 gave the best separation of CA

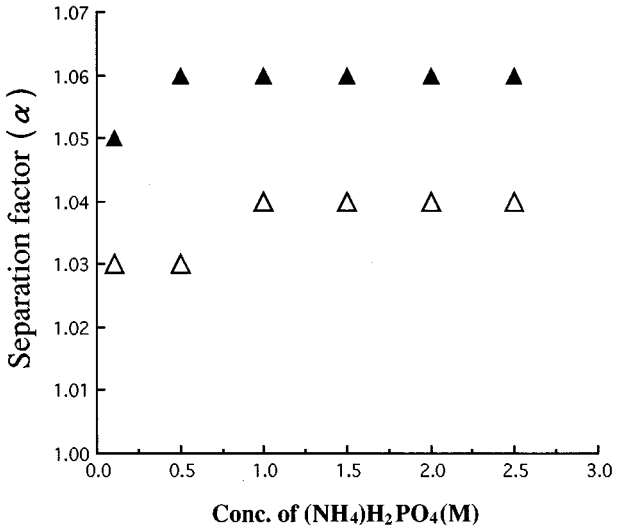

Figure 2. Effect of $NH_4H_2PO_4$ concentration in the mobile phase on separation factors (αs) of CAs. (▲*dl*-E; △*dl*-NE).

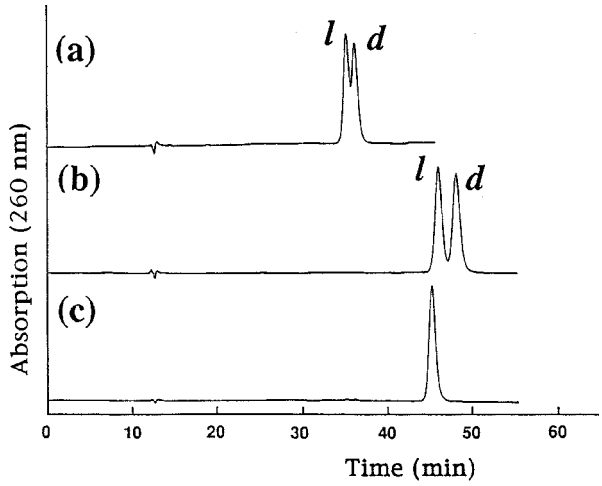

Figure 3. Chromatograms of E, NE and DA on Ultron ES-CD (150×6.0 mm, i.d.) connected in series using 2.5 M $(NH_4)H_2PO_4$ (pH 3.0) as the mobile phase. (a) *d*-/*l*-NE; (b) *d*-/*l*-E, (c) DA. The other HPLC conditions are described in the text.

enantiomers among the mobile phase tested, the following experiments were performed with the solution prepared by $(NH_4)H_2PO_4$–H_3PO_4 (pH 3.0).

As shown in Fig. 1, the increase of $(NH_4)H_2PO_4$ concentration in the mobile phase afforded the increase of capacity factors (*k*'). On the contrary, the separation factors (α s) of *dl*- E and -NE were kept constant above the concentration of 1.0 M (1.06 and 1.04, respectively, Fig. 2). Each E, NE enantiomer and DA eluted at different retention times with first elution of each *l*-enantiomer. In order to accomplish sufficient separation, a high concentration of $(NH_4)H_2PO_4$ in the mobile phase was necessary. Connecting two columns of Ultron ES-CD in series gave a superior enantiomeric separation of E and NE. Figure 3 shows the chromatograms of CAs on Ultron ES-CD connected in series using 2.5 M phosphate buffer (pH 3.0) as the mobile phase. E was well separated enantiomerically compared to NE, indicating hydrophobic interaction with a β-CD cavity occurred. Although a native β-CD has no enantioselectivity towards the separation of CA enantiomers in CE (Fanali, 1989), in HPLC with a native β-CD chiral stationary phase, an inclusion complexation with a β-CD cavity seemed to occur for the enantiomeric separation of CA. On the other hand, in using a phenylcarbomylated β-CD type column (Ultron ES-phCD), CA enantiomers were not separated at all under the same HPLC conditions. The difference of

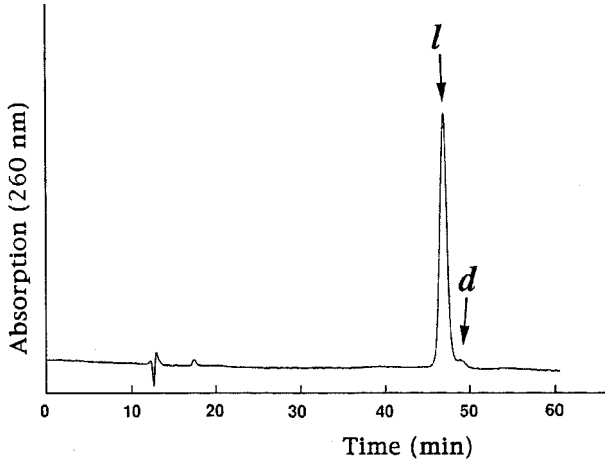

Figure 4. Chromatogram of Bosmin. The HPLC conditions are the same as in Fig. 1.

separation mechanism in HPLC from CE is unclear in the present study and should be clarified in the near future.

The present method was applied to determine the opitcal purity of E in an injection including *l*-E (Bosmin, Daiichi Pharmaceutical Co., Ltd). As shown in Fig. 4, the chromatogram shows the presence of 0.5% *d*-epinephrine in the injection. As the expiry date of the injection was 3 years later, the small percentage of *d*-E seems to be reasonable.

Compared with the separation methods of E enantiomer which were published previously, the present method has the advantages that no derivatization to diastereomer is required and elution time is reproducible. However, since UV absorption was adopted for the detection, a trace level of CA (femto mol) was difficult to detect under the present conditions. Thus, post-column derivatizaiton and chemiluminescence detection techniques should be required for the trace analysis of CAs in biological specimens, such as in plasma (Higashidate *et al.*, 1992; Prados *et al.*, 1994). The development of highly sensitive detection of CA enantiomers is now in progress.

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