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Enantiomeric separation of denopamine by capillary electrophoresis and high-performance liquid chromatography using cyclodextrins

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Abstract

Direct separation of enantiomers of denopamine was investigated by two separation methods. One is capillary zone electrophoresis (CZE) using cyclodextrins (CDs) (CD-CZE) and the other is high-performance liquid chromatography (HPLC) using CD immobilized chiral stationary phases (CD-CSPs). Enantiomers of denopamine were successfully resolved by employing heptakis (2,6-di-*O*-methyl)-*β*-cyclodextrin (DM-*β*-CD) and acetyl-*β*-cyclodextrin (AC-*β*-CD), and partially resolved with heptakis(2,3,6-tri-*O*-methyl)-*β*-cyclodextrin (TM-*β*-CD), hydroxypropyl-*β*-cyclodextrin (HP-*β*-CD) and *β*-CD polymer under acidic conditions. Separation of enantiomers of denopamine by HPLC was also successful with one of the CD-CSPs, where perphenylated *β*-CD (Ph*β*-CD) was immobilized. In CD-CZE, some polymeric additives, such as hydroxypropylmethylcellulose (HPMC) and polyvinylalcohol (PVA), and a coated capillary were used to improve the enantioseparation of denopamine. Method validations such as linearity, recovery and repeatability, etc., were investigated for HPLC, and the method was applied to the optical purity testing of the drug substances and in tablet form.

Keywords: Enantiomer separation; Capillary electrophoresis; Column liquid chromatography; Denopamine; Cyclodextrins

1. Introduction

Synthesis of chiral compounds and recognition of molecular chirality are important subjects, especially in the pharmaceutical industry, because stereochemistry can have a significant effect on the biological activity of a drug [1]. Further, it is necessary to develop a simple method for the optical purity determination of the drugs, because the antipode of a chiral drug is regarded as one of the impurities [2].

Denopamine, which is a new cardiotonic agent having phenetylethanolamine structure (Fig. 1), is an optically active drug. Therefore, it is required to develop a simple enantioseparation method for the optical purity determination of pharmacokinetic studies of the drug. For this purpose, chiral derivatization methods using 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl isothiocyanate (GITC) [3] or *S*(−)-*N*-1-(2-naphthylsulfonyl)-2-pyrrolidine carbonyl

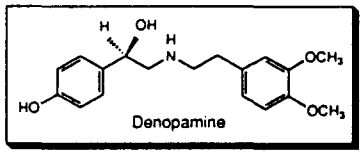

Fig. 1. The structure of denopamine.

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chloride (NSP-Cl) [4] are used because several chiral stationary phases (CSPs) gave no successful direct separation of the enantiomers of the drug.

Recently, new types of CSPs have been developed, and most of which are commercially available. In particular, CSPs using proteins (Protein-CSPs) such as bovine serum albumin, ovomucoid or avidin as chiral selectors have been demonstrated to be effective for the enantioseparation of a wide variety of compounds [5,6]. CSPs using cyclodextrin (CD) derivatives as chiral selectors (CD-CSPs) have also been developed [7,8]. These protein-CSPs and CD-CSPs are usable in the reverse-phase mode and have relatively long column life. The separattion of the enantiomers of denopamine by these CSPs was tried and was found to be successful by one of the new type CD-CSPs. The preliminary results were reported elsewhere [9].

Other than HPLC, the CE techniques have been demonstrated to be effective for the separation of enantiomers [10–12]. In particular, CD modified capillary zone electrophoresis (CD-CZE) is successful for a wide variety of the enantiomeric drugs [13,14]. The enantiomeric separation of denopamine was also successful by CD-CZE with DM-β-CD among six CDs employed [15].

In this work, we investigated and optimized the conditions for direct separation of enantiomers of denopamine in the HPLC method. The method validations such as linearity, recovery and repeatability were examined under the optimized conditions. The HPLC method was then applied to the optical purity testing of the drug substances and in tablet form, because of its high enantioselectivity and high precision. In the CE separation of enantiomers of denopamine, 14 CDs in total, which are ionic or electrically neutral, were employed. Further, the effects of polymer additives on the enantioseparation were investigated. The coated capillary was also employed to improve the enantioseparation of denopamine. Enantio-recognition of denopamine by CDs was discussed from the successful CDs in HPLC and CZE. The performance of the CE enantiomer separation of denopamine has been briefly described elsewhere [9].

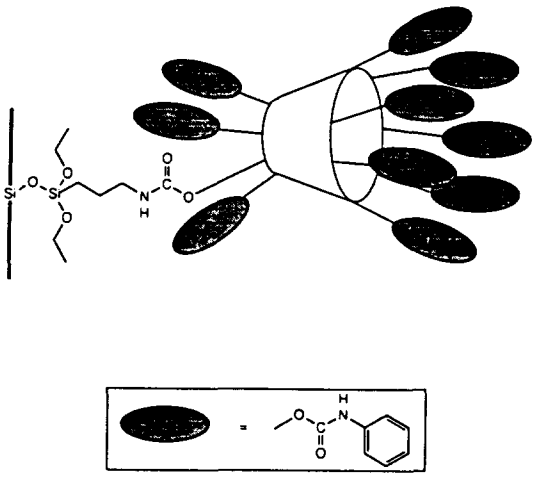

Fig. 2. Schematic illustration of Phβ-CD CSPs.

2. Experimental

2.1. CE instrument

CE experiment was performed on a Beckman P/ACE system 5510 equipped with a photodiode array detector (Beckman Instruments, Inc., Fullerton, CA, USA). Fused-silica capillary tubes with coating or non-coating (75 μm i.d., 57 cm total length, effective length 50 cm) were used for the separation. The polyacrylamide-coated capillary was prepared according to the Hjerten method [16]. The capillary was thermostated at 23°C with a liquid coolant. The applied voltage was held constant at 15 or 25 kV. The detection wavelength was adjusted to 210–220 nm and the detection point was at the cathode side. Sample introductions were performed by pressure mode (0.5 psi, 3–7 s) from the anode side. The instrument control and data collections were performed with a personal computer (COMPAQ ProLinea 4/33).

2.2. HPLC instrument

HPLC separation was carried out with the liquid chromatograph system of LC-6A or LC-10A (Kyoto, Japan, Shimadzu). Samples were introduced to the column by a Rheodyne Model 7125 loop injector or a Shimadzu SIL-10A auto injector. Peak integration was accomplished using a Chromatopac C-R5A data-processor (Shimadzu). The CSPs used were obtained from Shinwakako (Kyoto, Japan): β-CD immobilized CSP (ULTRON ES-CD, 5 μm, 6 mm i.d. × 150 mm) and β-CD having phenyl group immobilized CSP (Fig. 2) (ULTRON ES-PhCD, 5 μm, 6 mm i.d. × 150 mm). In the latter CSP, most of the

Table 1
CDs employed in the CE study

CDs	Substituted group	Degree of substitution	Solubility in water	Molecular mass (average or range)
Electrically neutral CDs				
α-CD	–	–	14.5%	973
HP-α-CD	Hydroxypropyl	≈3	>33%	(1130–1180)
β-CD	–	–	1.8%	1135
DM-β-CD	Methyl	14	>33%	1331
TM-β-CD	Methyl	21	>15%	1430
HP-β-CD	Hydroxypropyl	3 ~ 7	>33%	(1300–1500)
AC-β-CD	Acetyl	14 ~ 16	>10%	(1781)
Glu-β-CD	Glucosyl	1	≈80%	1297
Mal-β-CD	Maltosyl	1	≈100%	1459
β-CD polymer	(β-CD 3 ~ 50)[a]	–	>10%	(4000–50000)
γ-CD	–	–	23.2%	1297
HP-γ-CD	Hydroxypropyl	≈3	>33%	(1460)
Charged CDs				
β-CD phosphate	Phosphate	≈6	>33%	(1879)
γ-CD phosphate	Phosphate	≈6	>33%	(1997)

[a] The degree of polymerization through the epichlorohydrin.

hydroxy groups in β-CD (up to 20) were derivatized with phenylisocyanate [8]. The flow rate and column temperature were set at 1.0 ml min^{-1} and 40°C, respectively. The column effluent was monitored at 220 nm.

2.3. Reagents and materials

12 electrically neutral CDs, α-CD, hydroxypropyl-α-CD (HP-α-CD), β-CD, heptakis(2,6-di-*O*-methyl)-β-CD (DM-β-CD), heptakis-(2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD), hydroxypropyl-β-CD (HP-β-CD), 6-*O*-α-D-glucosyl-β-CD (Glu-β-CD), 6-*O*-α-D-maltosyl-β-CD (Mal-β-CD), acetyl-β-CD (AC-β-CD), β-CD polymer, hydroxypropyl-γ-CD (HP-γ-CD) and γ-CD were obtained from Nacalai Tesque (Kyoto, Japan) Cyclolab (Budapest, Hungary) and Wako Pure Chemicals (Osaka, Japan). Water soluble β-CD polymer was synthesized by condensation of β-CD molecules with epichlorohydrin. As ionic CDs, sodium β-CD phosphate and sodium γ-CD phosphate obtained from Cyclolab were used. Some characteristics of the CDs employed in the study are summarized in Table 1. Denopamine (R(−)-form), its racemate, Kargut® (denopamine tablets) and its placebo tablets were obtained from Tanabe Seiyaku Co. Ltd. (Osaka, Japan).

Acetonitrile (ACN), tetrahydrofuran (THF) of HPLC grade and methanol (MeOH) of analytical reagent grade were purchased from Katayama Kagaku Kogyo (Osaka, Japan). Acrylamide, *N,N,N′,N′*-tetramethylethylenediamide and ammonium peroxodisulfate were purchased from Wako. 3-Methacryloxypropyltrimethoxysilane was purchased from Shin-etsu Chemicals (Tokyo, Japan). All other reagents and solvents used were of analytical reagent grade from Katayama Kagaku Kogyo. Water was purified by a Milli-R0 60 water system (Millipore Japan, Tokyo, Japan).

The CD solutions were prepared by dissolving each CD in a 25 mM phosphate buffer solution (pH 2.5) containing 2 M urea. Urea was added to the buffer solution to increase the solubility of CDs, especially of β-CD [17]. The mobile phases for HPLC were prepared by mixing a phosphate buffer solution with an organic solvent. The solutions were passed through a membrane filter of 0.45 μm pore size (Gelman Science Japan, Tokyo) and degassed by sonication with a Branson MODEL B-2200 ultrasonic cleaner (Yamato, Tokyo, Japan) prior to use.

2.4. Sample preparations

The sample solutions of denopamine drug substances for HPLC were prepared in the mobile phase consisted of sodium dihydrogenphosphate (pH 4.6; 0.05 M)–ACN (75:25, v/v). About 10 mg of denopamine was weighed and dissolved in 20 ml of the mobile phase. 5 ml of the solution was pipetted into a 20 ml volumetric flask and the volume made up with the

Table 2
The enantiomer resolution by CE with CDs

CDs	Concentration	Buffer[a]	Migration time (min)		Rs
			R(−)-form	S(+)-form	
(None)		A	11.83	(11.83)	0
α-CD	20 mM	A	14.40	(14.40)	0
HP-α-CD	0.3%	A	16.07	(16.07)	0
β-CD	20 mM	A	16.28	(16.28)	0
DM-β-CD	20 mM	A	18.92	19.30	1.3
TM-β-CD	20 mM	A	13.50	13.70	0.8
HP-β-CD	0.3%	A	16.32	16.49	0.4
AC-β-CD	20 mM	A	19.92	20.43	1.4
Glu-β-CD	20 mM	A	14.40	(14.40)	0
Mal-β-CD	20 mM	A	14.45	(14.45)	0
β-CD polymer	0.3%	A	17.68	17.84	0.2
γ-CD	20 mM	A	14.50	(14.50)	0
HP-γ-CD	0.3%	A	14.37	(14.37)	0
β-CD phosphate	5 mM	B	6.42	6.60	0.4
γ-CD phosphate	5 mM	B	5.70	(5.70)	0

[a] A: 25 mM phosphate buffer of pH 2.5 containing 2 M urea; 15 kV; 220 nm; 23°C. B: 25 mM phosphate buffer of pH 9.0; 15 kV; 220 nm; 23°C. A non-coating capillary was used and the detection point is the cathode.

mobile phase. 20 μl of the solution was injected to HPLC. The extraction of denopamine from the tablets were carried out with the mobile phase. The tablets were weighed and ground. The resulting powder, equivalent to 10 mg of the drug substance according to the labeled amount, was weighed into a 20 ml volumetric flask, and the mobile phase was added to the volume for the extraction. The flask was shaken vigorously for 5 min and the solution was filtered with a membrane filter of 0.45 μm. 5 ml of the filtrate was pipetted into a 20 ml volumetric flask and the volume made up with the mobile phase. 20 μl of the solution was injected to HPLC. The final concentration of the sample solutions for the HPLC injection was 0.125 mg ml^{-1}. The stock solution of denopamine for CE was prepared in methanol at an approximate concentration of 1.0 mg ml^{-1}. The stock solution was diluted with water to a concentration of about 0.1–0.3 mg ml^{-1} for the CE injection.

3. Results and discussion

3.1. Separation of enantiomers of denopamine by CE with CDs

Denopamine is a new cardiotonic agent having a *N*-phenetylethanolamine structure and is active in its R-(−)-form. The optical purity testing of denopamine has been carried out by the derivatization method using GITC or NSP-Cl, because HPLC direct separation with protein-CSPs and some other CSPs was not successful. The direct separation of enantiomers of denopamine was first achieved by CD-CZE, where six CDs were examined [15]. We further investigated the enantiorecognition for other CDs. Fourteen CDs were employed in total. The concentration of CD was fixed at 20 mM or 0.3% for the electrically neutral CDs, and 5 mM for the charged CDs, although it is known that each CD has an optimal concentration for maximum enantiomer separation [18–20]. Urea was added to the buffer solution to increase the solubility of CDs, especially of β-CD. In some cases, it has been reported that urea addition improved the enantiomer separation [15,21]. A non-coating capillary was used for the separation. The results are summarized in Table 2. Denopamine was probably included in all CDs judging by the increase of the migration times. However, effective enantiorecognition of denopamine was observed in five electrically neutral CDs and one ionic CD, which are all β-type CDs. Enantiomer separation of denopamine by CD-CZE is shown in Fig. 3. DM-β-CD and AC-β-CD gave successful enantiseparation. TM-β-CD was also effective. Partial enantiorecognition of the enantiomers was observed in HP-β-CD and the β-CD polymer. In ionic CDs, alkaline conditions were employed. Partial enantioseparation was observed in β-CD phosphate.

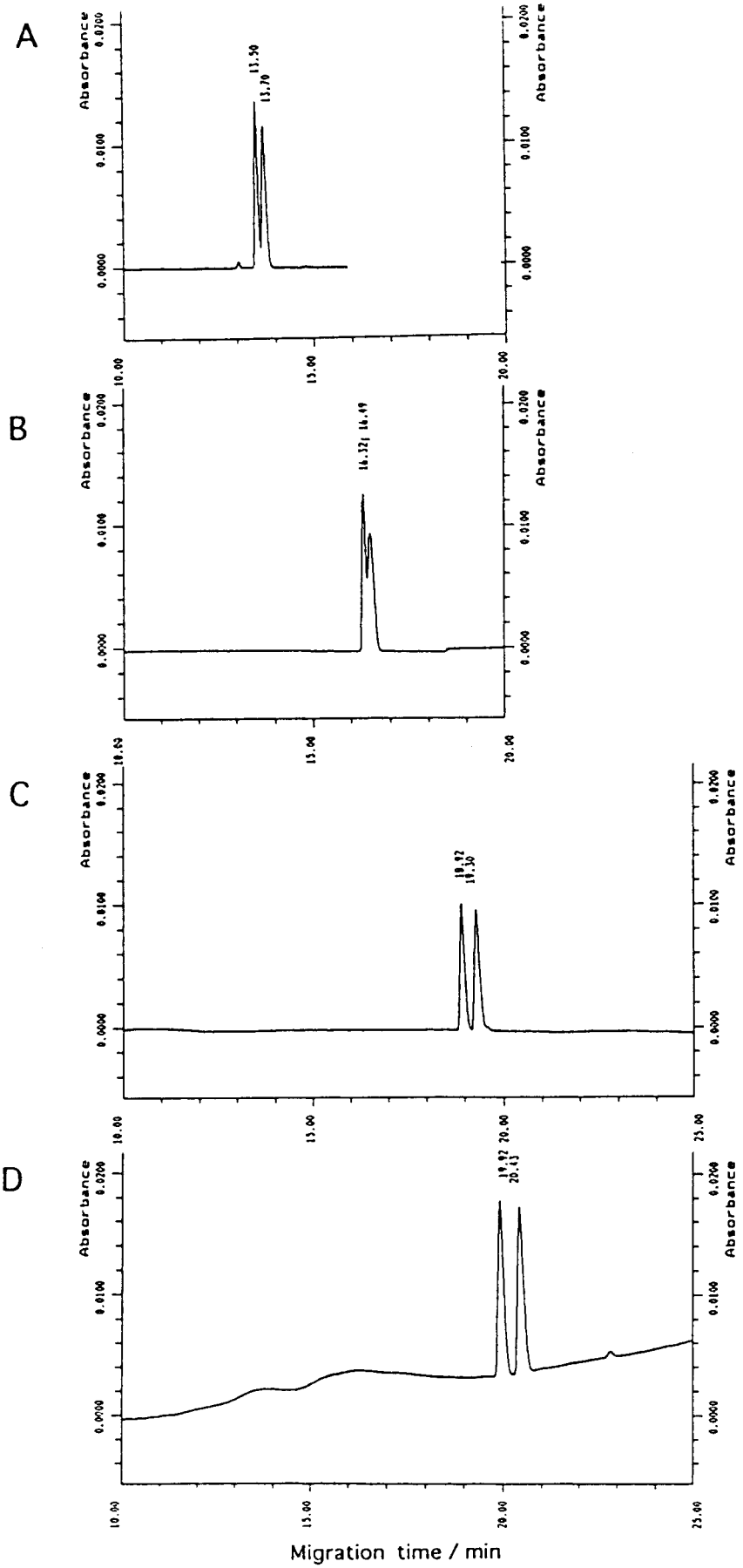

Fig. 3. Caption overleaf.

Table 2 indicates that β-type CD is essential for the enantiorecognition of denopamine. Further, hydrophobic groups such as acetyl or methyl groups in β-CD are probably very important for improving the enantioselectivity. Hydrophobic interaction between the solute and the hydrophobic groups in CDs entraps the solute inside the CD cage effectively, leading to the enhancement of enantiorecognition. Effective enantiorecognition in HPLC with Phβ-CD, mentioned below, also supports this conception. However, in the case of TM-β-CD, some distortion of the β-CD cage may reduce the enantiorecognition. The smaller decrease in the migration times in TM-β-CD than those in DM-β-CD or AC-β-CD suggests this interpretation. In β-CD phosphate, some ionic interaction may contribute to the enantiorecognition.

3.2. Effects of polymer additives and capillary coating on the enantioseparation of denopamine in CD-CZE

Denopamine has a second amino group, and its pK_a is 8.2 [22], i.e. denopamine has a cationic character at pH 2.5. There must be an ionic interaction between the solute and the capillary wall. There are several methods for preventing solute adsorption onto the capillary wall. One is to reduce the buffer pH at around 1–2 to prevent silanol group dissociation. Some additives have been successfully applied for the purpose [23,24]. Covering the capillary wall with a hydrophilic layer has also been effective [16,25]. Among the additives, some polymeric ones such as hydroxypropylmethylcellulose (HPMC) and polyvinylalcohol (PVA), and capillary coating by the Hjerten method [16] were investigated to improve the enantioseparation of denopamine in CD-CZE. 25 mM phosphate buffer of pH 2.5 containing 20 mM DM-β-CD and 2 M urea was employed as a buffer solution. The results are summarized in Table 3. The resolution (Rs) between the enantiomeric pairs was calculated by $(t2-t1)/(w1+w2)/2$, where $t1$, $t2$ are migration times, and $w1$, $w2$ are peak widths of the enantiomeric pairs. As in the separation of basic proteins, the improvement of Rs was obtained through the prevention of solute adsorption. Capillary coating was the most successful. Typical electropherograms are shown in Fig. 4. By adding such polymers in their given forms or in coated capillaries, it is natural that the electroosmotic flow can be reduced. The solute migration times and peak intensities were then adjusted to give almost the same values for comparison of the effect of these additives and coating, although the applied voltages had influence the theoretical plate numbers of the solute. The effect of applied voltage (V) on Rs can be estimated by $V^{1/2}$ [26], i.e. the contribution of the difference in the applied voltage to Rs is $25^{1/2}/15^{1/2}$ ($=1.3$). The improvement of Rs for the coated capillary was much larger than this value.

3.3. Separation of enantiomers of denopamine by HPLC with CD-CSPs

Recently, direct HPLC separation of the enantiomers of denopamine was achieved by perphenylated β-CD (Phβ-CD) immobilized CSPs (ULTRON ES-PhCD), although it was unsuccessful by β-CD immobilized CSPs (ULTRON ES-CD). Preliminary results were reported elsewhere [9]. As mentioned in CD-CZE, hydrophobic groups (i.e. phenyl groups) in β-CD probably contributed to the enantiorecognition of denopamine in HPLC. Perhaps $\pi-\pi$ interactions and hydrogen bonding through C=O, NH or OH also contributed to the enantiorecognition in Phβ-CD CSPs. No enantioseparation in natural β-CD immobilized CSPs supports this interpretation.

The mobile phase composition was optimized through the selection of an organic solvent, buffer concentration, buffer pH, etc. The effect of organic modifiers on the enantioseparation was investigated with MeOH, ACN and THF using a 50 mM phosphate buffer of pH 4.6. Among the three, THF showed the system peak, which is retained, and therefore we selected MeOH and ACN.

Fig. 3. Enantiomer separation of denopamine by CD-CZE using (A) TM-β-CD, (B) HP-β-CD, (C) DM-β-CD and (D) AC-β-CD. Conditions: buffer, 25 mM phosphate buffer of pH 2.5 containing 2 M urea and 20 mM each CD; separation tube, non-coating capillary 75 μm i.d. × 57 cm (effective length 50 cm); applied voltage, 15 kV; detection, 220 nm cathode side; temperature, 23°C.

Table 3
Effects of polymer additives and capillary coating on enantioseparation[a]

Capillary	Additives	Applied voltage	Migration time (min)		Rs
			R(−)-form	S(+)-form	
Non-coating	None	15 kV	20.01	20.39	1.0
Non-coating	0.05% PVA	15 kV	24.44	24.93	1.1
Non-coating	0.05% HPMC	25 kV	19.33	19.86	1.3
Coating	None	25 kV	22.72	23.28	1.8

[a] 25 mM phosphate buffer (pH 2.5) containing 20 mM DM-β-CD and 2 M urea was used; 220 nm (cathode side); 23°C.

The results in two organic solvents are summarized in Table 4. We selected ACN as an organic solvent from its low percentage requirement and sufficient enantiorecognition, although MeOH gave a larger Rs than ACN.

The effect of buffer concentration and buffer pH on the enantiorecognition were investigated with ACN as an organic solvent (buffer ACN = 75:25, v/v). The results are summarized in Tables 5 and 6, respectively. Phosphate buffer (pH 4.6) concentration (5–100 mM) were not so critical for the enantioseparation. However, the capacity factor (k') of the enantiomers largely changed with the buffer pH. The characteristics of denopamine change from ionic to electrically neutral, with an increase of pH values because of its pK_a of 8.2. More electrically neutral or hydrophobic solutes require larger percentages of an organic solvent in reverse-phase HPLC. With an increase of pH, the peak shapes or peak tailings (peak theoretical plate number) were also impaired, as well as larger retention. Hence, we used a 0.05 M potassium dihydrogenphosphate solution without pH adjustment (pH 4.6) as a buffer solution for simplicity. A typical HPLC chromatogram of the enantiomers of denopamine under the optimized conditions is shown in Fig. 5, where the active R-(−)-form eluted after the inactive form with Rs 2.7 within 9 min. Further, impurities and degradation products of active denopamine did not co-elute with *R*- and *S*-denopamine under these conditions.

Table 4
Effects of organic solvent of the mobile phase on enantioseparation[a]

Organic solvent	Percentage	$k'1$	$k'2$	α	Rs
Acetonitrile	20%	2.342	3.234	1.38	3.61
	25%	1.349	1.745	1.29	2.70
	30%	0.867	1.082	1.25	2.02
Methanol	50%	4.400	9.033	2.05	5.50
	60%	2.559	5.024	1.96	4.91
	70%	1.690	3.185	1.88	4.51

[a] Buffer: 0.05 M phosphate buffer of pH 4.6.

Table 5
Effects of buffer concentration on enantioseparation[a]

Buffer concentration (mM)	$k'1$	$k'2$	α	Rs
5	1.971	2.518	1.28	2.75
20	1.649	2.114	1.28	2.73
50	1.349	1.745	1.29	2.70
100	1.376	1.736	1.26	2.60

[a] Buffer: phosphate buffer of pH 4.6.

Table 6
Effects of buffer pH on enantioseparation[a]

Buffer pH	$k'1$	$k'2$	α	Rs
3.0	1.017	1.301	1.28	2.45
4.0	1.257	1.618	1.29	2.57
4.6	1.593	2.034	1.28	2.67
6.0	2.257	2.856	1.27	2.88
7.0	4.217	5.211	1.24	2.90

[a] Buffer: 0.05 M phosphate buffer.

3.4. Application of the HPLC method to the optical purity testing of denopamine

The optical purity testing of denopamine and those in tablets was investigated by the HPLC method. Some method validation data were examined under the optimized conditions. The reproducibilities of retention times, peak areas and peak heights of active enantiomer in six repeated injections were investigated using a SIL-10A auto injector. The RSD values obtained were all between 0.1 and 0.2%. The

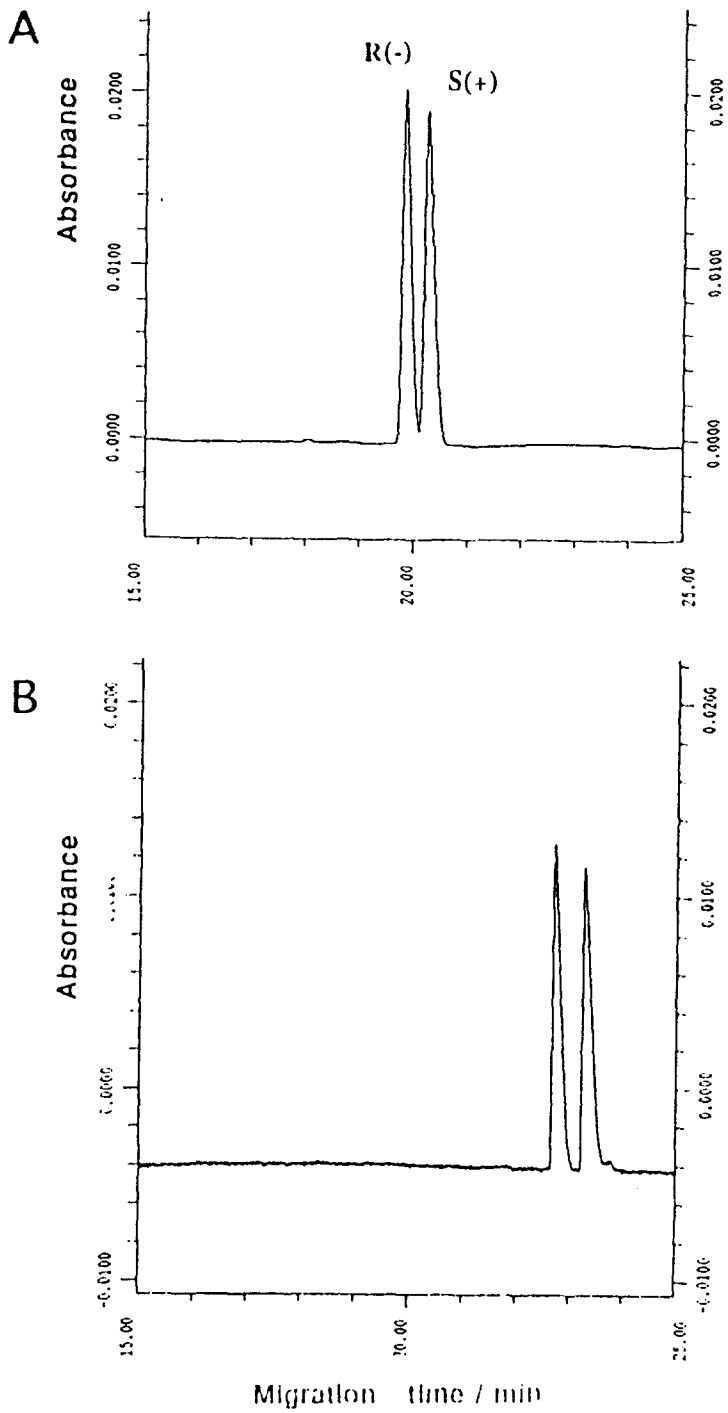

Fig. 4. Effects of capillary coating on enantiomer separation of denopamine in CD-CZE. (A) Non-coating capillary and (B) polyacrylamide-coated capillary. Buffer: 25 mM phosphate buffer of pH 2.5 containing 20 mM DM-β-CD and 2 M urea. Applied voltage: (A) 15 kV and (B) 25 kV. Other conditions are the same as in Fig. 3.

linearity of peak areas of the active enantiomer was investigted in the range 2.5–175 μg m^{-1}, which corresponds to 2–140% of the concentration of the sample solutions described in the experimental section. The obtained graph passed through the origin with the correlation coefficient $r = 0.999$. The results of recovery testing of the minor enantiomer in the range 0.1–2% were investigated by adding racemate to the active denopamine drug substance which contained 0.03% minor enantiomer, and are summarized in Table 7. Good linearity with

Table 7
Recovery testing of the minor enantiomer

Theoretical S-(+)-form	0.09%	0.45%	0.90%	1.33%	2.20%
Found S-(+)-form	0.11%	0.46%	0.89%	1.31%	2.14%

$r = 0.999$ and almost 100% recovery were obtained. A typical chromatogram of the denopamine drug substance spiked with about 0.1% of the S-(+)-form is shown in Fig. 6. A 0.1% level of the minor enantiomer can be easily detected by the method. The optical purity of denopamine tablets was also successfully determined because no interruption was observed from the placebo tablets. These results clearly indicate that the HPLC method is adaptable as a quality control method of the optical purity testing of denopamine drug substances and those in tablets. More than 0.1% of the minor enantiomer was not detected in all of the denopamine drug substances or denopamine tablets examined.

4. Conclusions

It was found that the enantiomers of denopamine were successfully separated by the CE method employing CDs or by the HPLC method using CD-CSPs. β-type CDs having hydrophobic groups were found to be effective for the enantiorecognition of denopamine. Optical purity testing by the HPLC method was demonstrated to be useful for the actual quality control method of the drug. 0.1% of the inactive enantiomer could be easily detected by the method. In the future, HPLC optical purity testing will be routinely used as a quality control method.

References

[1] I.W. Wainer, in D.E. Drayer (Eds.), Drug Stereochemistry, Marcel Dekker, New York, 1988, pp. 209–226.
[2] Anon, FDA's policy statement for the development of new stereoisomeric drugs, Chirality, 4 (1992) 338–340.
[3] H. Nishi, N. Fujimura, H. Yamaguchi, W. Jyomori and T. Fukuyama, Chromatographia, 30 (1990) 186–190.
[4] H. Nishi and Y. Kokusenya, Kagaku to Kogyo (Japanese), 65 (1991) 494–504.
[5] S.R. Narayanan, J. Pharm. Biomed. Anal., 10 (1992) 251–262.
[6] J. Haginaka, T. Murashima and C. Seyama, J. Chromatogr. A, 677 (1994) 229–237.
[7] S.C. Chang, G.L. Reid III, S. Chen, C.D. Chang and D.W. Armstrong, Trends Anal. Chem., 12 (1993) 144–153.

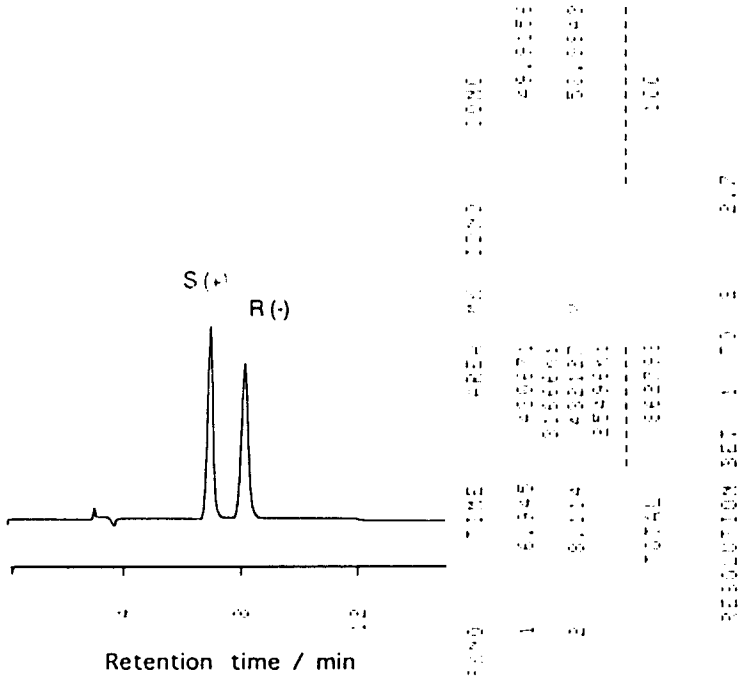

Fig. 5. Enantiomer separation by HPLC with Phβ-CD CSPs. Conditions: mobile phase, 0.05 M potassium dihydrogenphosphate (pH 4.6)–acetonitrile (75:25, v/v); flow rate, 1.0 ml min^{-1}; detection, 220 nm; column temperature, 40°C.

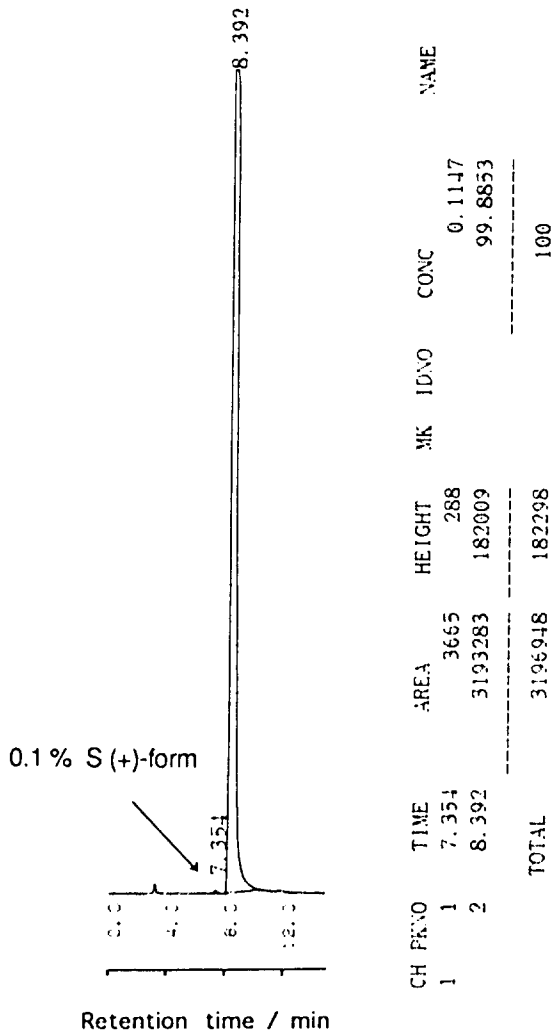

Fig. 6. Chromatogram of denopamine drug substance spiked with about 0.1% of the minor enantiomer. The conditions are the same as in Fig. 5.

[8] K. Nakamura, H. Fujima, H. Kitagawa, H. Wada and K. Makino, J. Chromatogr. A, 694 (1995) 111–118.
[9] H. Nishi, K. Nakamura, H. Nakai, T. Sato and S. Terabe, Chromatographia, 40 (1994) 638–644.
[10] T.J. Ward, Anal. Chem., 66 (1994) 633A–640A.
[11] M. Novotny, H. Soini and M. Stefansson, Anal. Chem., 66 (1994) 646A–6655A.
[12] S. Terabe, K. Otsuka and H. Nishi, J. Chromatogr. A, 666 (1994) 295–319.
[13] S. Fanali and F. Kilar, J. Capillary Electrophor. 1 (1994) 72–78.
[14] M. Heuermann and G. Blaschke, J. Chromatogr., 648 (1993) 267–274.
[15] H. Nishi, Y. Kokusenya, T. Miyamoto and T. Sato, J. Chromatogr., 659 (1994) 449–457.
[16] S. Hjerten, J. Chromatogr., 347 (1985) 191–198.
[17] D.Y. Pharr, Z.S. Fu, T.K. Smith and W.L. Hinze, Anal. Chem., 61 (1989) 275–277.
[18] S.A.C. Wren and R.C. Rowe, J. Chromatogr., 603 (1992) 235–241.
[19] S.A.C. Wren and R.C. Rowe, J. Chromatogr., 635 (1993) 113–118.
[20] Y.Y. Rawjee, D.U. Staerk and G. Vigh, J. Chromatogr., 635 (1993) 291–306.
[21] K. Otsuka and S. Terabe, Electrophoresis, 11 (1990) 982–984.
[22] H. Nishi, Y. Fujimoto, R. Honjyo, N. Tsumagari, M. Matsuo, T. Kakimoto and R. Shimizu, Iyakuhin Kenkyu (Japanese), 20 (1989) 991–1000.
[23] D. Belder and G. Schomburg, J. Chromatogr. A, 666 (1994) 351–365.
[24] M. Gilges, M.H. Kleemiss and G. Schomburg, Anal. Chem., 66 (1994) 2038–2046.
[25] M. Nakatani, A. Shibukawa and T. Nakagawa, J. Chromatogr. A, 661 (1994) 315–321.
[26] S. Terabe, T. Yashima, N. Tanaka and M. Araki, Anal. Chem., 60 (1988) 1673–1677.