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Thermodynamic study and separation mechanism of diltiazem optical isomers in packed-column supercritical fluid chromatography

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Abstract

Cellulose tris(4-chlorophenylcarbamate) coated on silica was used as a chiral stationary phase for the separation of diltiazem (a Ca-channel blocker) optical isomers in packed-column supercritical fluid chromatography. The effect of temperature on the separation of the *cis*- and *trans*-enantiomers was studied in detail. The Van't Hoff plots for the retention were found to be curves, while those for the selectivity were found to be linear. The chiral separation of *cis*-enantiomers was improved with a decrease in temperature, whereas that of *trans*-enantiomers was improved with an increase in temperature. To obtain a better understanding of the difference in the separations of *cis*- and *trans*-enantiomers, the temperature dependence of enantioselectivities was studied to determine the thermodynamic parameters $\Delta\Delta H°$, $\Delta\Delta S°$, and T_{iso}. The thermodynamic parameters revealed that the separation of *trans*-enantiomers was entropy-controlled in the range of temperature examined, whereas enthalpy-controlled separation was observed for *cis*-enantiomers. The separations of both *cis*- and *trans*-enantiomers, however, were enthalpy-controlled in normal-phase HPLC. The separations of the related compounds, 3-hydroxy isomers, were compared with those of diltiazem isomers. In addition, the differences in separation mechanisms between *cis*- and *trans*-enantiomers of diltiazem optical isomers are discussed by means of three-dimensional structures.

Keywords: Chiral stationary phases, SFC; Enantiomer separation; Retention mechanism; Thermodynamics; Diltiazem

1. Introduction

In chiral separation, the column temperature plays an important role not only in high performance liquid chromatography (HPLC), gas chromatography (GC), and capillary zone electrophoresis (CZE), but also in supercritical fluid chromatography (SFC) [1–9]. While column efficiency increases with temperature, the retention of solute is, in general, reduced due to the weakened interaction between the solute and the stationary phase. Especially in SFC, such an increase in column efficiency can often be observed rather than in HPLC.

There have been many papers discussing chiral separation by packed-column SFC (p-SFC) from a thermodynamic viewpoint. The density of the supercritical fluid changes with temperature and pressure and its solvent strength is related to the density.

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Frequently, however, in p-SFC using a binary or ternary mobile-phase fluid, the fundamental data concerning the density of the mobile-phase fluid is not sufficient and it is very difficult to determine or predict the density accurately. Many researchers have evaluated the thermodynamic parameters based on measurements carried out at constant pressure rather than at constant density [1–3] and have demonstrated highly interesting insights concerning chiral recognition. Smith et al. [1] studied the temperature dependence of chiral recognition using two racemates of very similar structures in SFC and HPLC. The selectivity of the benzoylamide derivatives increased, but that of the pentanoylamide derivatives decreased with increasing temperature. T_{iso}, at which enantiomers coelute, for the benzoylamide derivatives was below the used temperature range, and that for the pentanoylamide derivatives was above. They reported that the difference in chiral recognition should be ascribed to the difference in the $\Delta\Delta H^{\circ}$ values for the interaction between the analytes and the cellulosic stationary phase. Stringham and Blackwell [2,3] described 'entropically driven' separation in detail. The selectivity was a compromise between differences in enantiomeric binding enthalpy and disruptive entropic effects.

The cellulose chiral stationary phase has been successfully and widely applied not only to HPLC [10–12] but also to packed-column subcritical fluid chromatography (p-subFC) and/or p-SFC [13–15]. In a previous paper it was reported that four diltiazem optical isomers were separated by p-SFC with baseline resolution under higher column efficiency using a cellulosic chiral stationary phase. A method for the determination of small amounts of optical impurities in diltiazem hydrochloride was developed at levels down to ca. 0.05% [13].

In this research, cellulose tris(4-chlorophenylcarbamate), Chiralcel OF, was selected as the chiral stationary phase and the temperature dependence of the enantioselectivities for *cis*- and *trans*-diltiazem optical isomers and their related compounds were studied in detail under various p-subFC and p-SFC conditions from a thermodynamic point of view. The differences in separation mechanisms of *cis*- and *trans*-enantiomers of diltiazem optical isomers are discussed using the three-dimensional structures of diltiazem optical isomers obtained from X-ray analyses.

2. Experimental

2.1. Chromatographic equipment

The p-SFC system used is a modified HPLC system (Shimadzu, Kyoto, Japan) that has been described elsewhere [16]. For normal-phase HPLC, an LC-6A pump, a CTO-6A oven, an SPD-6A detector (Shimadzu) and a Rheodyne Model 7125 injector (Rheodyne, Cotati, CA, USA) fitted with a 20 μl sample loop were used. A Chiralcel OF column (250×4.6 mm I.D., Daicel Chemicals, Tokyo, Japan) was selected as the chiral stationary phase (CSP). The packing material was a silica-gel support (10 μm) coated with a polymer of cellulose tris(4-chlorophenylcarbamate).

2.2. Chemicals and reagents

The chemical structures of diltiazem optical isomers and related compounds are shown in Fig. 1. They were synthesized by Tanabe Seiyaku Co. (Osaka, Japan). They have two asymmetric carbons at positions 2 and 3. There are two isomers, *cis* and *trans*, depending on the relative positions of the substituents. Diltiazem hydrochloride is the *cis*-(2*S*,3*S*)-isomer. The hydroxyl form (3-hydroxy form) isomers were obtained by replacing the ester group of diltiazem isomers at position 3 with an hydroxyl group. The conformation energies of the three-dimensional molecular structures were minimized by molecular mechanics calculations (MM2) based on the structure obtained from X-ray analyses [17,18]. Liquid carbon dioxide (99.9%) was purchased from Kyoritu Shoji (Osaka, Japan). All other solvents, of HPLC- or analytical-grade, were obtained from Katayama Kagaku (Osaka, Japan).

2.3. Methods

For p-subFC and/or p-SFC, liquid carbon dioxide at a flow-rate of 2 ml/min was modified with an adequate amount of 2-propanol and diethylamine as the modifier and the additive, respectively. For p-subFC and/or p-SFC, the sample compounds were dissolved in ethanol at ca. 1 mg/ml before chromatographic operation. Detection was performed at 254 nm. The hold-up time was measured from the injection point to the top of the negative peak caused

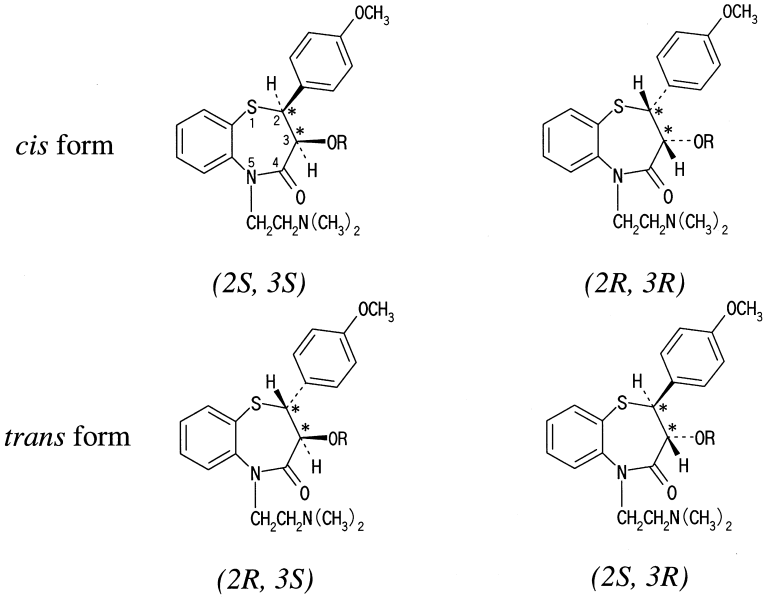

Fig. 1. Chemical structures of diltiazem optical isomers and related compounds. Compounds: diltiazem R=$COCH_3$, 3-hydroxy form R=H.

by ethanol. The HPLC separation was carried out using the same column and a mixture of *n*-hexane–2-propanol (1:1) containing 0.1% (v/v) diethylamine as the mobile phase at a flow-rate of 1 ml/min. Other conditions were the same as reported previously [19]. For HPLC, the sample compounds were also dissolved in ethanol at ca. 0.1 mg/ml before use. All data were obtained in duplicate.

3. Results and discussion

3.1. Influence of temperature on retention and selectivity

In chromatography, the thermodynamic relationship between the capacity factor (k) and the temperature can be expressed as

$$\ln k = -\Delta H^\circ/RT + \Delta S^\circ/R + \ln \beta \quad (1)$$

where ΔH° and ΔS° are the enthalpy and entropy changes in the partition process between the mobile and stationary phases, respectively, R is the gas constant (8.314 J mol^{-1} K^{-1}), T is the absolute temperature, and β is the phase ratio. The selectivity (α), the ratio of capacity factors for enantiomers, can be expressed as

$$\ln \alpha = -\Delta\Delta H^\circ/RT + \Delta\Delta S^\circ/R \quad (2)$$

where $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$ are the differences in the enthalpy and entropy changes for the enantiomers, respectively. In chiral separation, ΔH° and ΔS° can be considered as the sum of the chiral and achiral contributions: $\Delta H^\circ = \Delta H^\circ_{chiral} + \Delta H^\circ_{achiral}$, $\Delta S^\circ = \Delta S^\circ_{chiral} + \Delta S^\circ_{achiral}$. Only stereoselective interaction with the chiral stationary selector leads to a difference in retention; the achiral contributions to ΔH° and ΔS° should be the same for both enantiomeric pairs. Thus, $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$ should be ascribed to the difference in chiral contributions, i.e. $\Delta\Delta H^\circ_{chiral}$ and $\Delta\Delta S^\circ_{chiral}$, respectively. The temperature at which the enantiomers coelute is defined as T_{iso}, where the enthalpy and entropy terms are equivalent or balanced, and can be expressed by

$$T_{iso} = \Delta\Delta H^\circ/\Delta\Delta S^\circ \quad (3)$$

The influence of temperature on retention and selectivity of diltiazem optical isomers in p-subFC and/or p-SFC was investigated using a Chiralcel OF column and a mixture of CO_2–15% (v/v) 2-propanol containing 0.1% (v/v) diethylamine as the mobile

phase. The column temperatures were changed from 23 to 60°C under 180 kg/cm^2 constant outlet pressure. To obtain a better understanding of the chiral separation of diltiazem optical isomers thermodynamically, the values of the logarithms of k and α were plotted versus $1/T$, respectively. As shown in Fig. 2a, k of diltiazem optical isomers decreased, reached minimum values at 31 or 40°C, and increased with temperature. Such curvatures have often been confirmed not only in p-subFC and/or p-SFC, but also in HPLC [20,21]. On the other hand, plots of ln α versus $1/T$ yielded straight lines for both *cis*- and *trans*-enantiomers, as shown in Fig. 2b. The chiral separation of *cis*-enantiomers was improved by reducing the temperature, whereas that of *trans*-enantiomers was improved by increasing the temperature.

The thermodynamic parameters of *cis*- and *trans*-enantiomers calculated from the plots of ln α versus $1/T$ according to Eq. (2) are summarized in Table 1. They are completely different as expected from the plots of Fig. 2. The $\Delta\Delta H°$ and $\Delta\Delta S°$ of *cis*-enantiomers were -9500 J mol^{-1} and -25 J mol^{-1} K^{-1}, respectively, and those of *trans*-enantiomers were 4700 J mol^{-1} and 16 J mol^{-1} K^{-1}, respectively. The T_{iso} for *cis*- and *trans*-enantiomers were considerably different. The T_{iso} of *cis*-enantiomers was above the range of temperature examined; the separation of *cis*-enantiomers was enthalpy-controlled. On the other hand, the T_{iso} of *trans*-enantiomers was the lowest temperature in the range of temperature examined; the separation of *trans*-enantiomers was entropy-controlled.

Table 1
Thermodynamic parameters for diltiazem enantiomers by p-SFC[a]

Diltiazem isomer	$\Delta\Delta H°$ (J mol^{-1})	$\Delta\Delta S°$ (J mol^{-1} K^{-1})	T_{iso} (°C)
cis-Enantiomers	−9500	−25	104
trans-Enantiomers	4700	16	24

[a] SFC conditions: column, Chiralcel OF; mobile phase, CO_2–15% (v/v) 2-propanol containing 0.1% (v/v) diethylamine; flow-rate of CO_2, 2 ml/min; outlet pressure, 180 kg/cm^2; detection, 254 nm.

3.2. Comparison with HPLC

To compare the thermodynamic parameters in normal-phase HPLC with those in p-SFC, the temperature dependence of the retention and the selectivity for *cis*- and *trans*-enantiomers in normal-phase HPLC were studied in detail using the same Chiralcel OF column. Both plots of ln k and ln α versus $1/T$ gave straight lines. The thermodynamic parameters for HPLC are shown in Table 2. The separations for both *cis*- and *trans*-enantiomers were interesting-

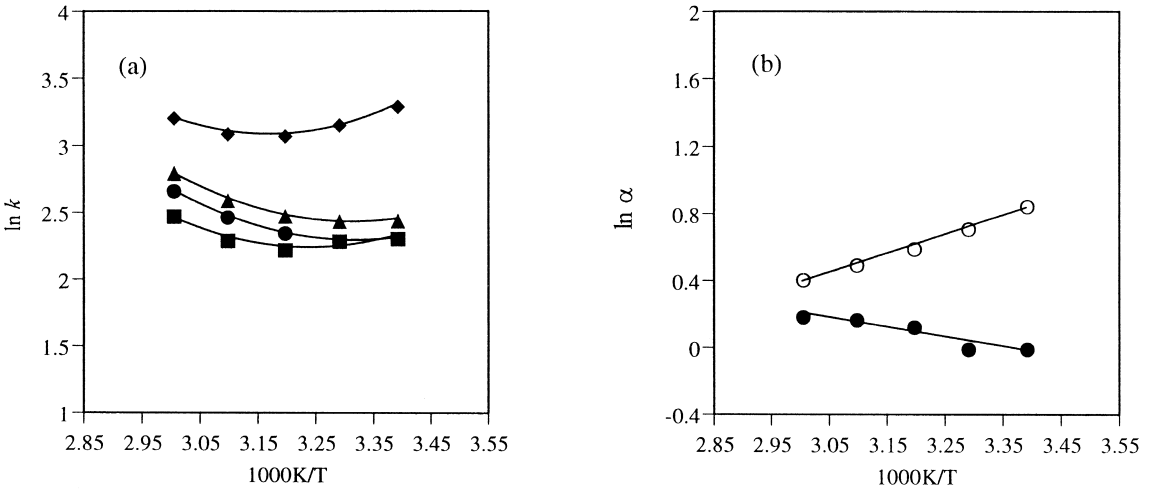

Fig. 2. Temperature dependence of retention and selectivity. p-SFC conditions: column, Chiralcel OF; mobile phase, CO_2–15% (v/v) 2-propanol containing 0.1% (v/v) diethylamine; flow-rate of CO_2, 2 ml/min; outlet pressure, 180 kg/cm^2; detection, 254 nm. (a) (♦) (2*S*,3*S*)-, (▲) (2*R*,3*R*)-, (●) (2*R*,3*S*)-, (■) (2*S*,3*R*)-; (b) (●) *trans*-enantiomers, (○) *cis*-enantiomers.

Table 2
Thermodynamic parameters for diltiazem enantiomers by HPLC[a]

Diltiazem isomer	$\Delta\Delta H°$ (J mol^{-1})	$\Delta\Delta S°$ (J mol^{-1} K^{-1})	T_{iso} (°C)
cis-Enantiomers	−8200	−21	111
trans-Enantiomers	−8000	−21	114

[a] HPLC conditions: column, Chiralcel OF; mobile phase, *n*-hexane–2-propanol (1:1) containing 0.1% (v/v) diethylamine; flow-rate, 1 ml/min; detection, 254 nm.

ly enthalpy-controlled. The T_{iso} of *cis*- and *trans*-enantiomers in HPLC were identical. These results reveal that the behavior of *trans*-enantiomers in p-SFC must be intrinsic in the supercritical fluid mobile phase.

3.3. Influences of separation parameters

The influence of pressure and the concentrations of modifier and basic additive on the separation of diltiazem optical isomers was investigated under various p-subFC and/or p-SFC conditions. The outlet pressure of the column was changed from 120 to 180 kg/cm^2, whereas the composition of the mobile phase was kept constant. The thermodynamic parameters were calculated from the plots of $\ln \alpha$ versus $1/T$. Increasing pressure had little significant influence on the thermodynamic parameters of both enantiomeric pairs, as shown in Table 3. The concentration of 2-propanol in the mobile phase was altered from 15 to 22.5% (v/v), keeping other conditions constant at 180 kg/cm^2 of pressure and 0.1% (v/v) diethylamine, and the temperature dependence of α was studied. The concentration of 2-propanol in the mobile phase also had little influence on the thermodynamic parameters, as shown in Table 3.

The influence of the diethylamine concentration on the separation of diltiazem optical isomers was investigated in the range from 0.05 to 0.5% (v/v) keeping other conditions constant: 180 kg/cm^2 pressure and 22.5% (v/v) 2-propanol. The k of *cis*- and *trans*-diltiazem optical isomers decreased with increase in diethylamine concentration. The k of the *trans*-(2*S*,3*R*)-isomer below 40°C decreased significantly with diethylamine concentration. The selectivity of *cis*-enantiomers was slightly improved with diethylamine concentration. On the other hand, for *trans*-enantiomers, the concentration of diethylamine in the mobile phase had a significant effect on the temperature dependence of the selectivity. The thermodynamic parameters for *cis*- and *trans*-enantiomers calculated from the plots of $\ln \alpha$ versus $1/T$ are shown in Table 4. The values of $\Delta\Delta H°$ and $\Delta\Delta S°$ of *cis*-enantiomers increased slightly from −9400 to −8100 (J mol^{-1}) and from −24 to −21 (J mol^{-1} K^{-1}), respectively, whereas those of *trans*-enantiomers markedly decreased from 7700 to 970 (J

Table 3
Influence of pressure and 2-propanol on thermodynamic parameters[a]

	dl-trans-Diltiazem		*dl-cis*-Diltiazem	
	$\Delta\Delta H°$ (J mol^{-1})	$\Delta\Delta S°$ (J mol^{-1} K^{-1})	$\Delta\Delta H°$ (J mol^{-1})	$\Delta\Delta S°$ (J mol^{-1} K^{-1})
Outlet pressure (kg/cm^2)[b]				
120	4900	17	−9000	−23
150	5700	19	−9300	−24
180	5500	18	−9100	−24
2-Propanol (%, v/v)[c]				
15.0	4800	16	−9100	−24
17.5	5200	18	−8800	−23
20.0	5500	18	−9100	−24
22.5	5400	18	−9100	−24

[a] SFC conditions: column, chiralcel OF; mobile phase, CO_2–20% (v/v) 2-propanol containing 0.1% (v/v) diethylamine; flow-rate of CO_2, 2 ml/min; outlet pressure, 180 kg/cm^2; detection, 254 nm.
[b] Mobile phase, CO_2–20% (v/v) 2-propanol containing 0.1% (v/v) diethylamine.
[c] Outlet pressure, 180 kg/cm^2.

Table 4
Effect of diethylamine concentration on thermodynamic parameters of diltiazem isomers by p-SFC[a]

Diethylamine	*trans*-Enantiomers			*cis*-Enantiomers		
(%, v/v)	$\Delta\Delta H^\circ$ (J mol^{-1})	$\Delta\Delta S^\circ$ (J mol^{-1} K^{-1})	T_{iso} (°C)	$\Delta\Delta H^\circ$ (J mol^{-1})	$\Delta\Delta S^\circ$ (J mol^{-1} K^{-1})	T_{iso} (°C)
0.05	7700	25	33	−9400	−24	110
0.1	5400	18	25	−9100	−24	112
0.2	5000	17	17	−8400	−22	115
0.5	970	5	−92	−8100	−21	114

[a] SFC conditions: column, Chiralcel OF; mobile phase, CO_2–22.5% (v/v) 2-propanol containing diethylamine; flow-rate of CO_2, 2 ml/min; outlet pressure, 180 kg/cm^2; detection, 254 nm.

mol^{-1}) and from 25 to 5 (J mol^{-1} K^{-1}), respectively, with diethylamine concentration.

In SFC, the amounts of CO_2 and modifier adsorbed to the stationary phase change remarkably near or at the critical point of the binary fluid [22,23]. The adsorbed basic additive prevents interactions between the solute and the residual silanol groups [24]. The modifier and additive in the mobile phase also play an important role in the stereoselectivity by binding to achiral sites near or at the chiral cavities of CSP, which alters the steric environment of these cavities [25,26]. Contrary to the behavior with p-SFC, the concentration of diethylamine had no influence on the chiral separation of diltiazem isomers in HPLC using a mixture of *n*-hexane/2-propanol as the mobile phase.

3.4. Influence of chemical structure on retention and selectivity

To obtain a better understanding of the difference in the temperature dependence of the enantioselectivities between *cis*- and *trans*-enantiomers, the chromatographic behaviors of the 3-hydroxy isomers which interacts with the stationary phase by stronger hydrogen bonding were compared with those of diltiazem isomers. Since the 3-hydroxy form of diltiazem hydrochloride is the precursor, the main degradation product and the metabolite, it is important to study its chromatographic behavior.

The elution order of the 3-hydroxy *trans*-enantiomers was reversed in the temperature range from 30 to 60°C, as seen in Fig. 3A. The separation of all isomers at 60°C is also shown in Fig. 3B. Table 5 summarizes the values of k and α for *cis*- and *trans*-enantiomers. All optical isomers could be separated simultaneously under these conditions. The values of both k and α of 3-hydroxy *cis*-enantiomers were almost the same as those for diltiazem isomers, although the 3-hydroxy form possessed a higher polar functional group. The k of 3-hydroxy *trans*-enantiomers, however, increased by a factor of 5 in comparison with diltiazem *trans*-enantiomers, whereas α showed the same values. These results indicate that the contributions of the side group at position 3 to the retention and selectivity were obviously different between *cis*- and *trans*-enantiomers.

The temperature dependence of the enantioselectivities for the 3-hydroxy form isomers was studied over the range from 25 to 60°C. The $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$ values of *trans*-enantiomers were 15 400 J mol^{-1} and 48 J mol^{-1} K^{-1}, respectively, and those of *cis*-enantiomers were −5500 J mol^{-1} and −13 J mol^{-1} K^{-1}, respectively. In comparison with the thermodynamic parameters of diltiazem optical isomers (Table 4) the $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$ values of 3-hydroxy *trans*-enantiomers increased remarkably; this resulted from the strong hydrogen bonding between the hydroxyl group of the 3-hydroxy form and the CSP.

The three-dimensional structure of each isomer provides a deeper insight into the differences in the separation mechanism between *cis*- and *trans*-enantiomers. The structures of *trans*-(2*R*,3*S*)- and *cis*-(2*S*,3*S*)-isomers of diltiazem and their 3-hydroxy compounds obtained from X-ray analyses are shown in Fig. 4. The chiral active site formed by the 4-methoxyphenyl group, the ester group and the carbonyl group of the solute interacts with the cellulose chiral stationary phase. These models reveal that there is a difference in the steric structure at

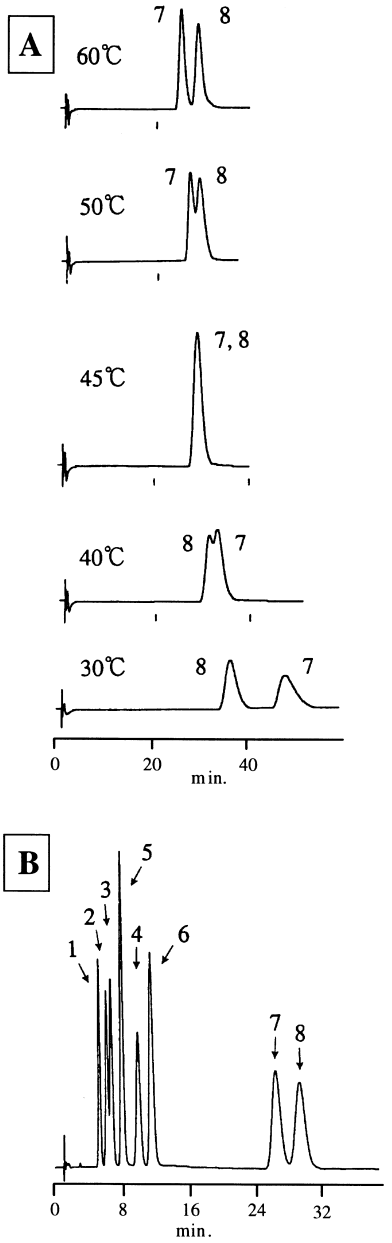

Fig. 3. Chromatograms of diltiazem and 3-hydroxy form isomers by p-SFC. p-SFC conditions: column, Chiralcel OF; mobile phase, CO_2–22.5% (v/v) 2-propanol containing 0.1% (v/v) diethylamine; flow-rate of CO_2, 2 ml/min; outlet pressure, 180 kg/cm^2; temperature, (A) 30, 40, 45, 50 and 60°C, (B) 60°C; detection, 254 nm. Peaks: 1=(2*S*,3*R*)-dialtiazem, 2=(2*R*,3*S*)-diltiazem, 3=(2*R*,3*R*)-diltiazem, 4=(2*S*,3*S*)-diltiazem, 5=(2*R*,3*R*)-3-OH, 6=(2*S*,3*S*)-3-OH, 7=(2*S*,3*R*)-3-OH, 8=(2*R*,3*S*)-3-OH.

the chiral active site between the *cis*- and *trans*-enantiomers. *Cis*- and *trans*-enantiomeric pairs form globular and planar-type structures, respectively. Thus, the *trans*-enantiomers have an open space at the chiral active site, and form a steric environment where the side group at position 3 can interact with the CSP effectively. On the other hand, there is a more closed steric environment at the chiral active site of the *cis*-enantiomers owing to hindrance by the 4-methoxyphenyl group, and the interaction of the side group at position 3 with the CSP is suppressed. Such differences in the steric environment can successfully explain the observed retention behavior of *cis*- and *trans*-enantiomers of diltiazem and its 3-hydroxy form.

Recently, Wada et al. [27] reported a local excess density about substituted benzene compounds in supercritical CO_2 based on FT-IR spectroscopy. They found that the local density of CO_2 about substituents on the aromatic ring was different from that around the aromatic ring itself and was also strongly dependent on the kind of substituent. The steric environment around a functional group should have some influence on the local density of solvents around it. The environments around the chiral active sites including the phenyl groups of diltiazem isomers and the phenylcarbamate group of the CSP in p-SFC may be different from those in HPLC. Taking into account the three-dimensional structures of diltiazem and the 3-hydroxy form enantiomers, the difference in the configuration of the aromatic ring at position 2 or the substituents at position 3 between *trans*- and *cis*-enantiomers may lead to a difference in the degree of solvation. Therefore, the behaviors of *cis*- and *trans*-enantiomeric pairs are different in chiral separations by p-SFC.

4. Conclusions

The temperature dependence of k and α for diltiazem optical isomers in p-SFC was studied and compared with those in normal-phase HPLC. The separation of *cis*-enantiomers was enthalpy-controlled, whereas the separation of *trans*-enantiomers was entropy-controlled in p-SFC in the temperature range examined. In HPLC, both *cis*- and *trans*-enantiomers showed enthalpy-controlled separation.

Table 5
Influence of structure on separation[a]

Compound	*trans*-Enantiomers			*cis*-Enantiomers		
	$k_{(2S,3R)}$	$k_{(2R,3S)}$	α	$k_{(2R,3R)}$	$k_{(2S,3S)}$	α
Diltiazem	4.32	5.32	1.23	5.83	9.11	1.56
3-Hydroxy form	24.26	27.78	1.15	6.56	10.04	1.53

[a] SFC conditions: column, Chiralcel OF; mobile phase, CO_2–22.5% (v/v) 2-propanol containing 0.1% (v/v) diethylamine; flow-rate of CO_2, 2 ml/min; outlet pressure, 180 kg/cm^2; temperature, 60°C; detection, 254 nm.

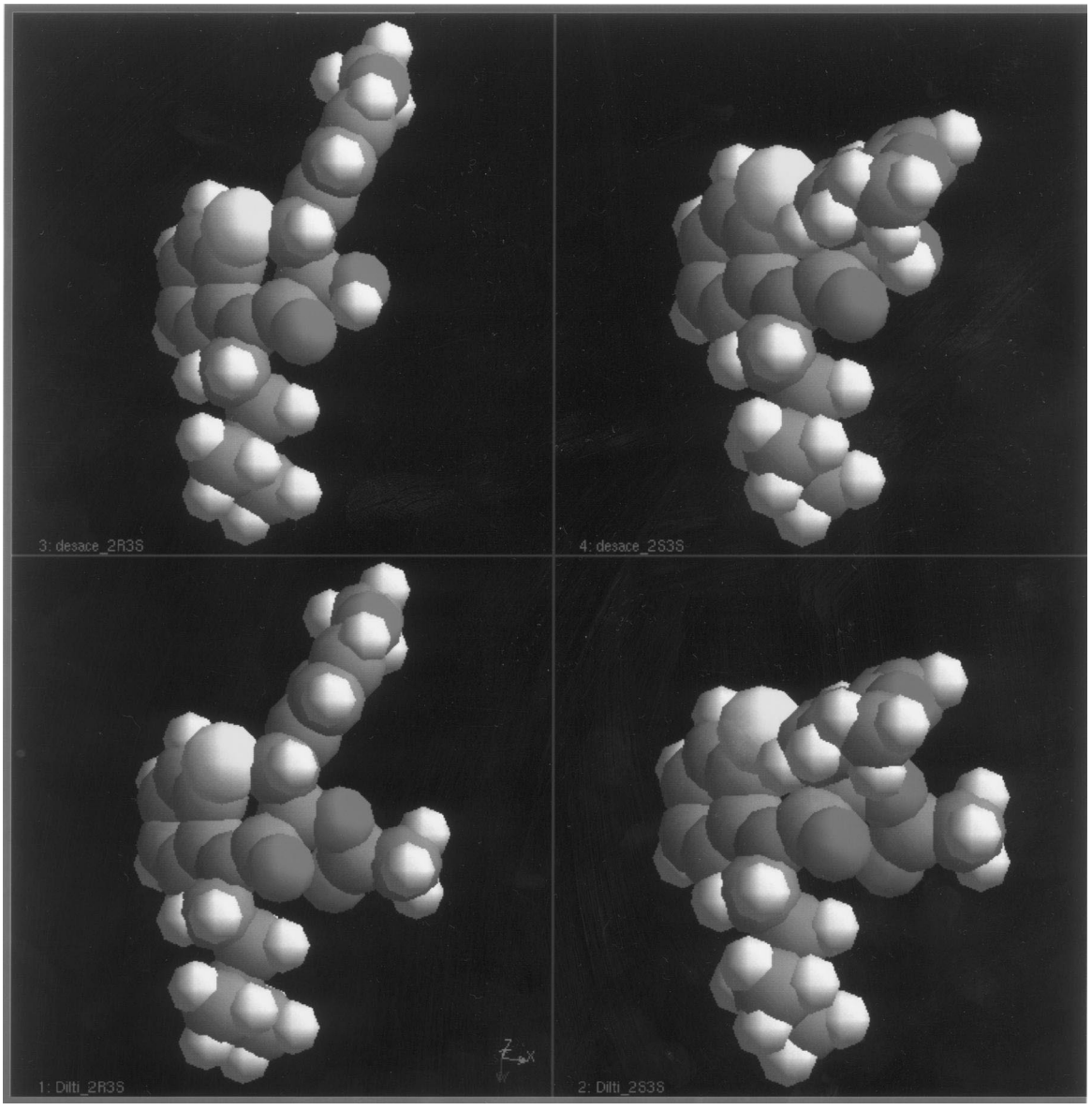

Fig. 4. Molecular structures of diltiazem and its 3-hydroxy optical isomers. Lower right, *cis*-(2*S*,3*S*)-diltiazem isomer; lower left, *trans*-(2*R*,3*S*)-diltizem isomer; upper right, *cis*-(2*S*,3*S*) 3-hydroxy isomer; upper left, *trans*-(2*R*,3*S*) 3-hydroxy isomer.

For *trans*-enantiomers, the diethylamine concentration in the mobile phase had a significant effect on the temperature dependence of the selectivity, α; this was never encountered in HPLC. To obtain a better understanding of the difference in the temperature dependence for enantioselectivity between *cis*- and *trans*-enantiomers, the separations of the 3-hydroxy isomers were compared with those of diltiazem isomers. In addition, the retention and enantioselectivity of diltiazem and its 3-hydroxy isomers were related to the difference in the steric environments at the chiral active sites of *cis*- and *trans*-enantiomers. Clearly, *cis*- and *trans*-enantiomeric pairs formed globular and planar-type structures, respectively. Although the side group at position 3 contributed to the strong retention of *trans*-enantiomers, such a contribution was suppressed by the steric hindrance of the 4-methoxyphenyl group for *cis*-enantiomers. Considering the local density, the steric environments around the phenyl groups of diltiazem isomers and phenylcarbamate groups of the CSP in p-SFC may be different from those in HPLC.

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