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Journal of Chromatography A, 785 (1997) 185–193

JOURNAL OF CHROMATOGRAPHY A

Chiral resolution of four optical isomers of diltiazem hydrochloride on Chiralcel columns by packed-column supercritical fluid chromatography

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Abstract

The optical isomers of diltiazem hydrochloride Ca-channel blocker were separated by packed-column sub- and/or supercritical fluid chromatography (p-SubFC and/or p-SFC) on a column of modified cellulose coated onto silica, using a mixture of carbon dioxide with a polar modifier and additive. The effect of parameters (Chiralcel columns, modifiers, additive, pressure and temperature) was investigated systematically, and the interactions between the diltiazem optical isomers and chiral stationary phase were discussed. Four optical isomers of diltiazem hydrochloride were achieved with baseline resolution on the Chiralcel OD column within 8 min. To determine three optical impurities in diltiazem hydrochloride, the precision, accuracy, linearity and detection limit were investigated, and the results were sufficiently acceptable. In addition, the resolutions in p-SFC were compared with those in high-performance liquid chromatography.

Keywords: Diltiazem hydrochloride; Enantiomer separation; Mobile phase composition; Stationary phases, SFC; Pharmaceutical analysis

1. Introduction

Diltiazem hydrochloride, (2*S*,3*S*)-3-acetoxy-2,3-dihydro-2-(4-methoxyphenyl)-5-(2-dimethylaminoethyl)-1,5-benzothiazepine-4(5*H*)-one monohydrochloride (shown in Fig. 1), is a benzothiazepine-type Ca-antagonist developed originally by Tanabe Seiyaku Co.. It is widely used over the world for the treatment of angina pectoris, variant angina and essential hypertension, which are attributable to the Ca-antagonistic action [1,2]. Diltiazem hydrochloride has asymmetric carbons at positions 2 and 3. There are two isomers, *cis* and *trans*, depending on the relative positions of the substituents. Each isomer also has optical isomers, *d*- and *l*-isomers. Diltiazem hydrochloride is a *d-cis*-(2*S*,3*S*)-isomer. It is known that, in general, the determination of the optical impurity in the drug is very important from the efficacy and safety point of view. The methods of the separations for optical isomers of diltiazem hydrochloride by the high-performance liquid chromatography (HPLC) have already been reported, using the formation of diastereomeric derivatives with a chiral reagent [3,4], and the direct resolution with a normal-phase chiral column [5] and a ovomucoid-conjugated reversed-phase column [6,7]. Four optical isomers of diltiazem hydrochloride were resolved on a Chiralcel OF column simultaneously even though the separation showed a poor efficiency, which took about 30 min in analysis time [7].

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0021-9673/97/$17.00
PII S0021-9673(97)00623-7

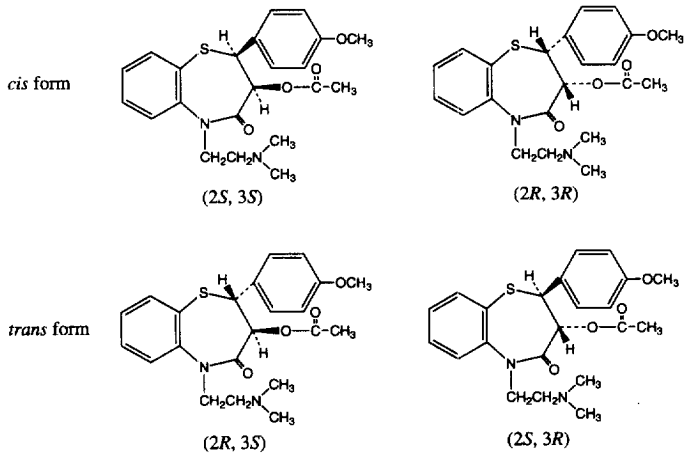

Fig. 1. Chemical structures of diltiazem optical isomers.

Sub- (SubFC) and/or supercritical fluid chromatography (SFC) has been used as a powerful chiral separation technique because of the advantage in the mobile phase which shows a low viscosity, and a high diffusion coefficient. Since Mourier et al. [8] introduced it in the first report, many researchers have shown the merits of chiral separation in packed column (p-)SubFC and/or p-SFC. Many studies using the derivatized cellulose packings in p-SubFC and/or p-SFC have been reported as well as in HPLC [9–18]. So far, only a few papers have been published on the determination of optical impurity at low level [12,18].

The purpose of this work is to investigate the effect of paramerters (Chiralcel columns, modifiers, additive, pressure and temperature) on the chiral resolution of diltiazem optical isomers, systematically, to optimize the conditions with a high column efficiency, and to study the determination of three optical impurities at lower level in diltiazem hydrochloride in p-SFC. In addition, the separation in p-SFC was compared with that in HPLC.

2. Experimental

2.1. Apparatus

The apparatus used was described in the previous report in detail [19]. A high-performance liquid chromatograph was modified for p-SFC operation. Carbon dioxide was passed into a LC-6A pump (Shimadzu, Kyoto, Japan) through a cooling-bath. The pump head was cooled to −10°C to improve pump efficiency. Polar modifiers were added by use of LC-9A pump (Shimadzu), and mixed with carbon dioxide in a dynamic mixer. Samples were introduced onto the column via Rheodyne 8125 injector fitted with a 5 μl-sample loop (Rheodyne, Cotati, CA, USA). The column was kept in the oven CTO-6A (Shimadzu) at a constant temperature. The SPD-6A photometric detector (Shimadzu) set at 254 nm was used, which was equipped with a high pressure flow cell. The sub- and/or supercritical conditions was maintained by the manual back-pressure regulator (TESCOM, Elk River, MI, USA) connected in-line after the detector and it was kept at ca. 40°C by a heating-unit. All results were recorded with a Chromatopak C-R5A integrator (Shimadzu). Since the additive was added in the modifier volumetrically, the volume was shown by % (v/v) in the modifier all through this study. LC-6A pump, CTO-6A oven, SPD-6A detector and a Rheodyne model 7125 injector fitted with a 20 μl-sample loop were used in the normal-phase HPLC.

2.2. Chemicals and materials

The liquid carbon dixide (99.9%) was purchased from Kyoritu Shoji (Osaka, Japan). The chemical structures of *dl-cis*-diltiazem and *dl-trans*-diltiazem isomers are shown in Fig. 1. They were synthesized

by Tanabe Seiyaku Co. (Osaka, Japan). HPLC-grade methanol, ethanol, 2-propanol and all other chemicals of analytical-reagent grade were obtained from Katayama Kagaku (Osaka, Japan).

2.3. Columns

The following Chiralcel column (250×4.6 mm I.D., Daicel Chemicals, Tokyo, Japan) was commercially available. The packings of Chiralcel OD, OC and OF columns were coated onto a silica support with polymer of cellulose tris(3,5-dimethylphenyl carbamate), cellulose tris(phenylcarbamate) and cellulose tris(4-chlorophenyl carbamate), respectively. The particle size was 10 μm.

2.4. Methods

In p-SubFC and/or p-SFC, samples were dissolved in ethanol at ca. 1–5 mg/ml before the chromatographic operation. In HPLC, samples were dissolved in ethanol at ca. 0.13 mg/ml before use. The hold-up time was measured from the injection point to the top of the peak caused by ethanol. The HPLC condition reported previously [7] was applied except for the mobile phase on Chiralcel OD which was optimized the separation; *n*-hexane–2-propanol (9:1) containing 0.1% (v/v) diethylamine.

All data were obtained in duplicate except for the determination of three optical impurities in diltiazem hydrochloride.

3. Results and discussion

3.1. Effect of chiral columns

To investigate the effect of a column on a chiral resolution of the optical isomers, 3 types of CSPs with the different substituents of cellulose tris(phenyl carbamate) derivatives were used. As shown in Fig. 2, four optical isomers were completely resolved on Chiralcel OD and OF columns, however, *d*- and *l-trans* diltiazem hydrochloride were not resolved on a Chiralcel OC column under the conditions examined. Although the selectivity (α) and resolution (R_S) of both *trans* and *cis* enantiomers on the Chiralcel OD column were smaller than those on the Chiralcel OF column, the theoretical plate numbers (N) were higher by a factor of about 3. The α, R_S and N values on Chiralcel OD column were 1.13, 1.65 and 5895 for *trans* enantiomer, respectively, and 1.17, 2.27 and 6137 for *cis* enantiomer, respectively, and those on the Chiralcel OF column were 1.19, 1.72 and 2051 for *trans* enantiomer, respectively, and 1.63, 4.85 and 2022 for *cis* enantiomer, respectively. The higher α value on the Chiralcel OF column might result from increasing the degree of interaction betwee the solute and CSP caused by longer retention. The elution of each solute on the Chiralcel OD column, however, was faster by a factor of about 4–5 and four optical isomers eluted with the baseline resolution within 8 min. Therefore, the Chiralcel OD column was selected in order to obtain the higher column efficiency on the chiral resolution of diltiazem hydrochloride in p-SFC.

Okamoto et al. [20,21] described that the most important adsorbing sites for chiral discrimination on phenylcarbamate derivatives of CSP in HPLC were probably the polar carbamate. The π–π interaction of phenyl groups on CSPs might be less important than the interaction of the carbamate for the chiral recognition ability. The retention and chiral discrimination of four optical isomers of diltiazem hydrochloride on the columns in p-SFC can be explained by assuming the interactions. That is, the 4-methoxylphenyl groups of the solute interact with the phenyl groups on CSPs. The ester group of the carbonyl oxygen of the solute interact with the NH proton on CSPs by the hydrogen-bonding. This interaction contributes significantly not only to the retention but also to the chiral discrimination of diltiazem optical isomers. The contribution of a steric effect of the derivatized cellulose on the chiral separation should be also considered [20,22].

3.2. Effect of modifiers and additive

The effect of the alcoholic nature, methanol, ethanol and 2-propanol, on the chiral separation of the optical isomers was studied using the Chiralcel OD column under the other constant conditions. As shown in Table 1, although the N values obtained on 2-propanol were smaller than those on others, the α and R_S of both *trans* and *cis* enantiomers were the highest. This might be caused by longer retentions

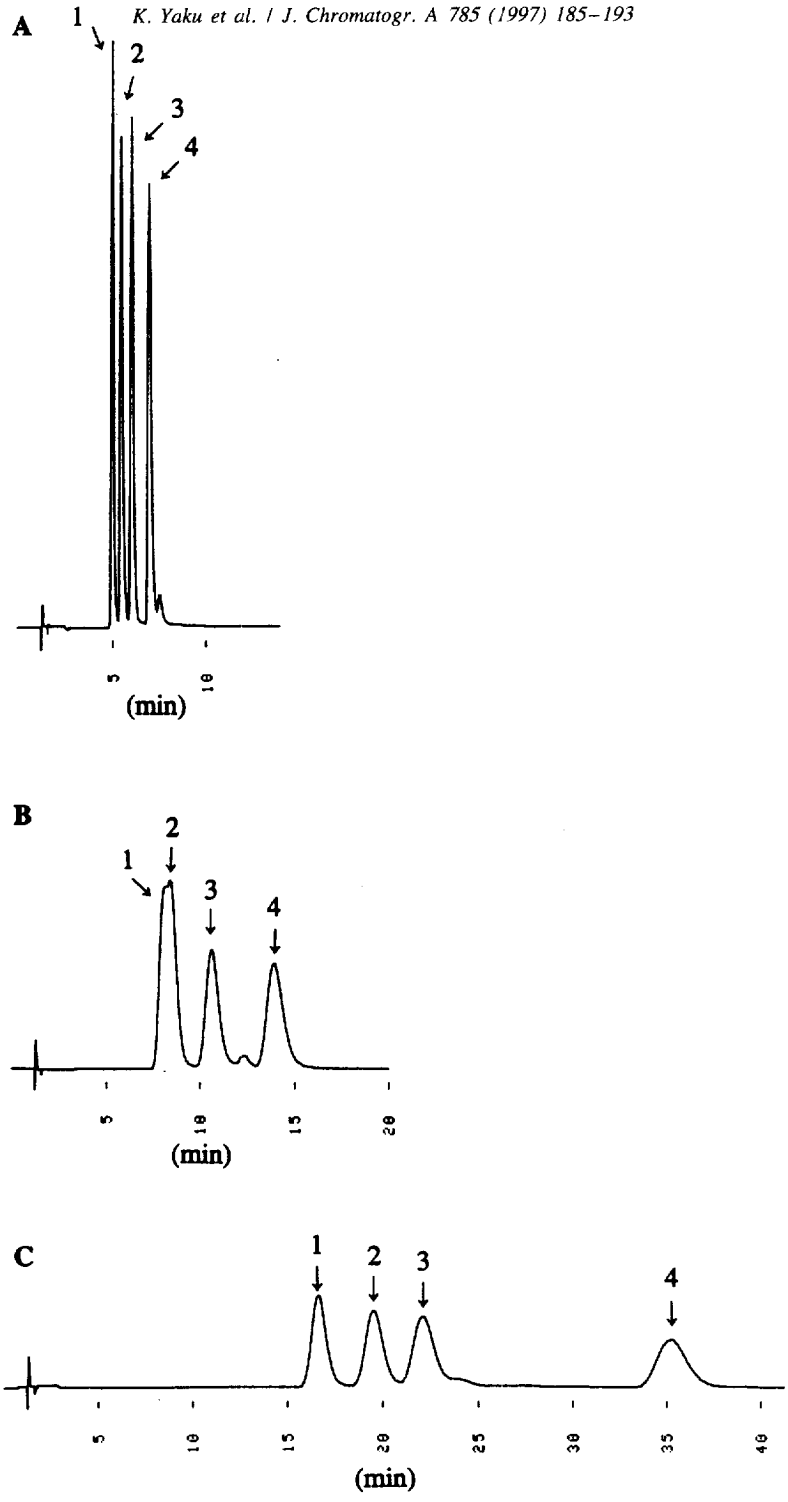

Fig. 2. Effect of columns on chiral separations by SFC. (A) Chiralcel OD, (B) Chiralcel OC, (C) Chiralcel OF. SFC conditions: mobile phase CO_2–13% (v/v) isopropanol containing 0.5% (v/v) diethylamine, flow rate of CO_2 2 ml/min, outlet pressure 180 kg/cm^2, temperature 50°C, detection 254 nm. Peaks: 1 *l-trans* isomer, 2 *d-trans* isomer, 3 *l-cis* isomer, 4 *d-cis* isomer.

Table 1
Effect of alcoholic natures on chiral separation of diltiazem optical isomers by SFC

Modifiers	*dl-trans* diltiazem					*dl-cis* diltiazem				
	k_1	k_2	α	R_s	N	k_1	k_2	α	R_s	N
Methanol	3.0	3.3	1.09	1.33	7570	3.8	4.3	1.13	1.97	7280
Ethanol	3.2	3.7	1.15	2.05	6640	4.1	4.7	1.16	2.37	6830
2-Propanol	5.2	6.4	1.24	2.98	4840	6.7	8.3	1.23	2.92	4840

SFC conditions: mobile phase CO_2–9% (v/v) modifier containing 0.5% (v/v) diethylamine, column Chiralcel OD, flow rate of CO_2 2 ml/min, outlet pressure 180 kg/cm^2, temperature 50°C, detection 254 nm.
k_1: Capacity factor of first-eluted enantiomer (*l*-isomer). k_2: Capacity factor of second-eluted enantiomer (*d*-isomer).

on 2-propanol and hence a greater interaction with CSP. In this p-SFC condition, 2-propanol was selected considering the stability of CSP on the polar organic solvent such as methanol, as recommended by the manufacturer.

Sandra et al. [11] mentioned that more important than the nature of the modifier was its concentration for a chiral separation. The effect of the concentrations of 2-propanol in the mobile phase were investigated in the range of 4.8–16.7% (v/v). As shown in Table 2, the retention of each solute decreased by a factor of about 7 with an increase in 11.9% (v/v) of 2-propanol concentration, and the selectivity and resolution also decreased. It was assumed that decreasing the number of stereoselective solute–CSP interactions was caused by the preferential solvation of chiral sites by the polar modifier [23], and that was due to a decrease in column efficiency by lower diffusion in a mobile phase containing more modifier [11]. On the other hand, the selectivity of the geometric isomer, *d-trans* and *l-cis* isomers, was improved from 1.00 to 1.14 in the range of 2-propanol concentration used, and the baseline resolution (R_S>1.5) was obtained in more than 13.0% (v/v) 2-propanol. The N values increased from 2500 to 6400 in the range of 2-propanol concentration. Considering these observations, 13.0% (v/v) of 2-propanol concentration was selected as a polar modifier.

To improve the peak shape by the deactivation of the active sites on the silica support, the low concentration of diethylamine was added in the modifier as the basic additive, and the effect of the additive was investigated in the range of 0.2–1.0% (v/v) diethylamine in the modifier. The concentration of diethylamine had little effect on the selectivity, but slightly changed the resolution. Considering the stability of CSP on the basic mobile phase, only 0.5% (v/v) diethylamine in the modifier was selected in the further work.

3.3. Effect of pressure

The outlet pressure was varied from 120 to 200 kg/cm^2 for the investigation of the chiral separations. The retentions of each solute decreased by a

Table 2
Effect of modifier concentrations on chiral separation of diltiazem optical isomers by SFC

Isopropanol (%, v/v)	*dl-trans* diltiazem					*dl-cis* diltiazem					Geometric isomer[a]	
	k_1	k_2	α	R_s	N	k_1	k_2	α	R_s	N	α	R_s
4.8	14.2	19.0	1.33	3.54	3330	19.0	24.5	1.29	2.81	2500	1.00	–
9.1	5.3	6.3	1.20	2.53	5100	6.9	8.4	1.22	2.75	4730	1.09	1.21
13.0	3.1	3.5	1.14	1.83	5850	3.9	4.7	1.20	2.55	6070	1.11	1.63
16.7	2.1	2.3	1.11	1.38	6260	2.6	3.1	1.17	2.22	6390	1.14	1.72

SFC conditions: column Chiralcel OD, mobile phase CO_2 – isopropanol containing 0.5% (v/v) diethylamine, flow rate of CO_2 2 ml/min, outlet pressure 180 kg/cm^2, temperature 50°C, detection 254 nm.
k_1: Capacity factor of first-eluted enantiomer (*l*-isomer). k_2: Capacity factor of second-eluted enantiomer (*d*-isomer).
[a] The values of separations between *d-trans* and *l-cis* isomers.

factor of about 2.2 with increase in pressure. Increasing pressure had little significant effect on the selectivity. Each resolution of both *trans* and *cis* enantiomers, however, were slightly changed; 1.65–1.80 for *d,l-trans* isomers and 2.31–2.59 for *d,l-cis* isomers, and the maximum values were 1.80 in 140 kg/cm^2 for *d,l-trans* isomers and 2.59 in 160–180 kg/cm^2 for *d,l-cis* isomers. The *N* values increased from 4600 to 6400 in proportion as the pressure increased. Therefore, the optimum column pressure was 180 kg/cm^2 for this p-SFC.

3.4. Effect of temperature

The effect of temperature is significantly important for the chiral separation in p-SubFC and/or p-SFC [24,25]. The temperature was varied from 23 to 60°C under the other conditions fixed. As shown in Table 3, the *N* values of each isomer tended to increase with increasing temperature, and the selectivity of *d,l-trans* isomers slightly increased and that of *d,l-cis* isomers obviously decreased. The different behaviors of the resolutions could be observed clearly in the change of the temperature; increase in 0.74 for *d,l-trans* isomers and decrease in 0.33 for *d,l-cis* isomers. It is likely that better resolution of *d,l-trans* isomers is obtained in the supercritical region even though precise critical parameters of this condition are not available, whereas that of *d,l-cis* isomers is obtained in the subcritical reagion. There were the quite different temperature dependencies of the resolutions between *trans* and *cis* enantiomers. The optimum temperature was 50°C where all optical isomers showed the complete baseline resolutions (R_S>1.5) with high efficiency. In a normal-phase HPLC, little attention has been focused on this parameter from a practical point of view. This is mainly due to the use of a combustible organic solvent such as *n*-hexane. It is one of the significant merits in p-SFC that carbon dioxide is used as a main mobile phase constituent. And there is also an effect of changing density with temperature not present in normal-phase HPLC.

3.5. Determination of three optical isomers in diltiazem hydrochloride

The precision, accuracy, linearity and limit of detection were examined under the above optimized conditions. The precision was investigated by performing six replicate injections of a solution of *d-cis*-diltiazem hydrochloride containing ca. 1.0% of *l-trans*, *d-trans* and *l-cis* isomers. The mean results were 0.92, 0.90 and 0.93% for *l-trans*, *d-trans* and *l-cis* isomers, respectively, and the relative standard deviations were from 1.5 to 1.8%. The accuracy and linearity were also evaluated by the spiked tests at low level of the optical impurities. The methods were determined by injecting spiked solutions containing 0.05, 0.10, 0.39 and 1.01% of *l-trans*, *d-trans* and *l-cis* isomers in *d-cis*-diltiazem hydrochloride. The differences of the found values of *l-trans*, *d-trans* and *l-cis* isomers against the added amounts were −0.07–+0.01%, −0.07–+0.03% and −0.05–+0.02%, respectively. The results showed good accuracy in this determination. The linearities in the range examined were good, as follows; the linear equation $y=0.02+0.915x$ ($r=1.000$) for *l-trans* iso-

Table 3
Effect of temperature on chiral separation of diltiazem optical isomers by SFC

°C	*dl-trans* diltiazem					*dl-cis* diltiazem				
	k_1	k_2	α	R_s	N	k_1	k_2	α	R_s	N
23	2.1	2.3	1.11	1.01	3800	2.8	3.6	1.29	2.60	3430
30	2.3	2.5	1.11	1.25	4980	3.0	3.8	1.26	2.70	4320
40	2.5	2.8	1.13	1.44	5970	3.3	4.0	1.22	2.56	5410
50	3.0	3.4	1.13	1.65	6530	3.9	4.6	1.19	2.59	6150
60	3.9	4.4	1.13	1.75	6390	4.9	5.8	1.17	2.27	6310

SFC conditions: column Chiralcel OD, mobile phase CO_2 – 13% (v/v) isopropanol containing 0.5% (v/v) diethylamine, flow rate of CO_2 2 ml/min, outlet pressure 180 kg/cm^2, detection 254 nm.
k_1: Capacity factor of first-eluted enantiomer (*l*-isomer). k_2: Capacity factor of second-eluted enantiomer (*d*-isomer).

mers, $y=0.03+0.903x$ ($r=1.000$) for *d-trans* isomers and $y=0.02+0.936x$ ($r=0.999$) for *l-cis* isomers, where y=observed response and x=theoretical response. The limit of detection and quantification of the three optical impurities in *d-cis*-diltiazem hydrochloride was 0.05% (12.5 ng at a signal-to-noise ratio of 2). Typical chromatograms of diltiazem hydrochloride spiked with ca. 0.05 and 1.0% of the three optical isomers are shown in Fig. 3. The determination of optical impurities in diltiazem hydrochloride by the peak area percentage method exhibited acceptable precision, accuracy, linearity and detection limit.

The optimized method was performed for the determination of three optical impurities in recent bulk drugs, and they were not detected in them; not more than ca. 0.05% by the area percentage method.

3.6. Comparison with HPLC

In HPLC, the optical isomers of diltiazem hydrochloride was resolved with the mobile phase of *n*-hexane/2-propanol/diethylamine on the Chiralcel OF column [7]. The separations of four optical isomers on the Chiralcel OD and OF column obtained in p-SFC (shown in Fig. 2) were compared with those in HPLC. As shown in Fig. 4, *d-trans* and *l-cis* isomers were not resolved on the Chiralcel OD column in HPLC, though they were the geometric isomer. On the Chiralcel OF column, all isomers achieved the baseline separations in both modes, but the elution orders of *d-trans* and *l-cis* isomers on p-SFC and HPLC were different. The N values obtained in p-SFC were higher by a factor of about 2–3.8 in comparison with that in HPLC; 2022–6137

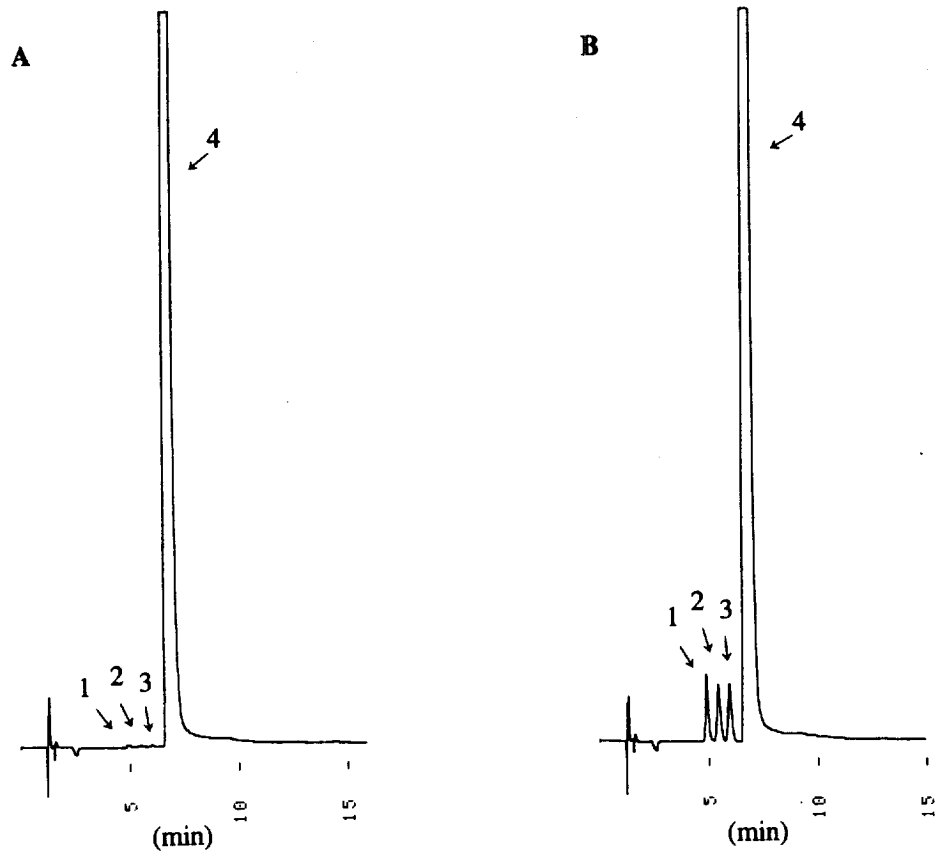

Fig. 3. Chromatograms of diltiazem spiked with three optical isomers (A) 0.05%, (B) 1.0%, SFC conditions and peaks as in Fig. 2.

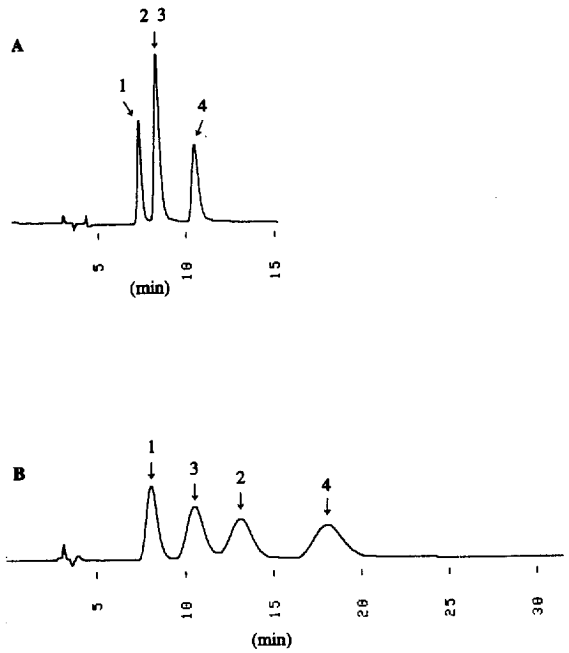

Fig. 4. Chiral separations of diltiazem optical isomers by HPLC. (A) Chiralcel OD, (B) Chiralcel OF. HPLC conditions: mobile phase *n*-hexane–isopropanol (A 9:1, B 1:1) containing 0.1% (v/v) diethylamine, flow rate 1 ml/min, temperature 30°C, detection 254 nm and peaks as in Fig. 2.

in p-SFC and 539–3223 in HPLC. It revealed that higher efficiency could be obtained in p-SFC rather than in HPLC, especially on the Chiralcel OD column, concerning the chiral separation of diltiazem hydrochloride.

4. Conclusions

Based on the evaluation of the effect of the parameters in p-SubFC and/or p-SFC, the method of the chiral resolution and the determination of the optical impurities in diltiazem hydrochloride were optimized. The resolutions were compared with those in HPLC.

The different behaviors of the chiral resolution in diltiazem optical isomers were observed concerning the change in the modifier concentration and temperature. The resolutions of both *trans* and *cis* enantiomers were lost with increasing 2-propanol concentration, while that of the geometric isomer, *d-trans* and *l-cis* optical isomers, improved. The resolutions of both *trans* and *cis* enantiomers showed the reversed behavior according to change in the temperature: the former exhibited better resolution in the supercritical condition (though a critical temperature of the mobile phase used is unknown) and the latter exhibited it in the subcritical condition. In p-SFC, four optical isomers achieved the baseline resolution within 8 min under the higher column efficiency on the Chiralcel OD column, but, in HPLC, *d-trans* and *l-cis* isomers did not resolve on the column. These results indicate that, in p-SFC, the chiral recognition mechanism is different between *trans* and *cis* enantiomers, owing mainly to the different three dimensional structures of *trans* and *cis* optical isomers.

The good precision, accuracy, linearity and detection limit permitted the determination of small amounts of the optical impurities at levels down to ca. 0.05%, and the applicability for a quality control and a pharmacokinetic study as a rapid method under the high column efficiency can be expected.

References

[1] M. Chaffman, R.N. Brodgen, Drugs 29 (1985) 387–454.
[2] M.T. Buckley, S.M. Grant, K.L. Goa, D. McTavish, E.M. Sorkin, Drug 39 (1990) 757–806.
[3] R. Shimizu, K. Ishii, N. Tsumagari, M. Tanigawa, M. Matsumoto, J. Chromatogr. 253 (1982) 101–108.
[4] R. Shimizu, T. Kakimoto, K. Ishii, Y. Fujimoto, H. Nishi, N. Tsumagari, J. Chromatogr. 357 (1986) 119–125.
[5] K. Ishii, K. Banno, T. Miyamoto, T. Kakimoto, J. Chromatogr. 564 (1991) 338–345.
[6] H. Nishi, N. Fujimura, H. Yamaguchi, T. Fukuyama, J. Chromatogr. 633 (1993) 89–96.
[7] K. Ishii, K. Minato, N. Nishimura, T. Miyamoto, T. Sato, J. Chromatogr. A 686 (1994) 93–100.
[8] P.A. Mourier, E. Eliot, M.H. Caude, R.H. Rosset, Anal. Chem. 57 (1985) 2819–2823.
[9] P. Petersson, K.E. Markides, J. Chromatogr. A 666 (1994) 381–394.
[10] C.R. Lee, J.-P. Porziemsky, M.-C. Aubert, A.M. Krstulovic, J. Chromatogr. 539 (1991) 55–69.
[11] A. Kot, P. Sandra, A. Venema, J. Chromatogr. Sci. 32 (1994) 439–448.
[12] P. Biermanns, C. Miller, V. Lyon, W. Wilson, LC·GC 11 (1993) 744–747.
[13] L. Siret, P. Macaudiere, N. Bargmann-Leyder, A. Tambute, M. Caude, E. Gougeon, Chirality 6 (1994) 440–445.

[14] K. Anton, J. Eppinger, L. Frederiksen, E. Francotte, T.A. Berger, W.H. Wilson, J. Chromatogr. A 666 (1994) 395–401.
[15] M.Z. Wang, M.S. Klee, S.K. Yang, J. Chromatogr. B 665 (1995) 139–146.
[16] R.W. Stringham, Chirality 8 (1996) 249–257.
[17] K.G. Lynam, E.C. Nicolas, J. Pharm. Biomed. Anal. 11 (1993) 1197–1206.
[18] R.W. Stringham, K.G. Lynam, C.C. Grasso, Anal. Chem. 66 (1994) 1949–1954.
[19] K. Yaku, K. Aoe, N. Nishimura, T. Sato, F. Morishita, J. Chromatogr. A 773 (1997) 277–284.
[20] Y. Okamoto, M. Kawashima, K. Hatada, J. Chromatogr. 363 (1986) 173–186.
[21] E. Yashima, Y. Okamoto, Bull. Chem. Soc. Jpn. 68 (1995) 3289–3307.
[22] N. Bargmann-Leyder, A. Tambute, M. Caude, Chirality 7 (1995) 311–325.
[23] P. Macaudiere, M. Caude, R. Rosset, A. Tambute, J. Chromatogr. Sci. 27 (1989) 583–591.
[24] R.J. Smith, D.R. Taylor, S.M. Wilkins, J. Chromatogr. A 697 (1995) 591–596.
[25] R.W. Stringham, J.A. Blackwell, Anal. Chem. 68 (1996) 2179–2185.