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Direct chromatographic resolution of four optical isomers of diltiazem hydrochloride on a Chiralcel OF column

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Abstract

Diltiazem hydrochloride is a calcium channel blocker which is administered as a single enantiomer. The direct resolution of four optical isomers of diltiazem hydrochloride was studied on both normal- and reversed-phase chiral HPLC columns. Four optical isomers of diltiazem hydrochloride were completely resolved on a Chiralcel OF column within 30 min. This chiral resolution was applied to determine three optical impurities that might be present in diltiazem hydrochloride bulk drug and its tablets. The determination of three optical impurities in the diltiazem hydrochloride bulk drug and those in tablets was successfully achieved at levels down to 0.05% by the area percentage method.

1. Introduction

HPLC for the determination of the optical purity of chiral substances is now a well developed technique. The past 15 years have given rise to a profusion of new developments such as chiral stationary phases (CSPs), derivatizing reagents and chiral mobile phase additives, all of which have proved effective in achieving chiral separations.

Four major approaches involving the separation of optical isomers by HPLC are (1) direct separation on a CSP [1–3], (2) derivatization with an achiral reagent and separation on a CSP [4,5], (3) direct separation on an achiral stationary phase with the use of a chiral mobile phase additive [6,7] and (4) derivatization with a chiral reagent and separation of the resulting diastereomers using an achiral support [8–10].

Diltiazem hydrochloride, *d*-3-acetoxy-*cis*-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(*p*-methoxyphenyl)-1,5-benzothiazepine-4-(5*H*)-one hydrochloride, is a calcium channel blocker widely used in the treatment of angina pectoris, hypertension and supraventricular tachyarrythmias [11,12]. Diltiazem hydrochloride has asymmetric carbons at positions 2 and 3. Two isomers, *cis* and *trans*, exist, depending on the relative positions of the substituents at these positions. Each isomer also has optical isomers, the *d*- and *l*-forms. Diltiazem hydrochloride is the *d-cis*-(2*S*,3*S*)-isomer.

The efficacy of the *d-cis*- and *l-cis*-diltiazem hydrochloride was investigated. It was found that only *d-cis*-diltiazem hydrochloride had a coronary vasodilating effect. That is, the coronary

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vasodilating effect of *dl-cis*-diltiazem hydrochloride was further dependent on the absolute configuration of position 2 and position 3, and only *d-cis*-diltiazem hydrochloride was active [13]. On the other hand, it was found that the stimulating effect for electroencephalography of *l-cis*-diltiazem hydrochloride was twice as strong as that of *d-cis*-diltiazem hydrochloride [14]. Therefore, it is very important for ensuring efficacy and safety to determine the optical impurity in diltiazem hydrochloride bulk drug and its tablets.

Enantiomeric resolution of diltiazem hydrochloride was first achieved by the formation of diastereomeric derivatives with a chiral reagent and subsequent separation by HPLC [9,10]. Recently, the direct chiral resolution of enantiomers of diltiazem hydrochloride has been reported using a normal-phase chiral HPLC column (Chiralcel OC) [15] and a reversed-phase chiral HPLC column (ovomucoid-conjugated column) [16]. However, the direct resolution of the four optical isomers of diltiazem hydrochloride has not been reported.

A number of cellulose-based HPLC chiral stationary phases and an ovomucoid-conjugated column, Ultron ES-OVM, have been developed, and are now commercially available. The resolution of the four optical isomers of diltiazem hydrochloride was examined on four cellulose tris(phenylcarbamate) derivatives having as substituents on the phenyl groups none, 4-CH_3, 3,5-$(CH_3)_2$ and 4-Cl and on an ovomucoid-conjugated column.

This paper describes a method for the simultaneous determination of three optical impurities in diltiazem hydrochloride bulk drug and its tablets on a Chiralcel OF column.

2. Experimental

2.1. Apparatus

The HPLC equipment consisted of a Shimadzu (Kyoto, Japan) LC-9A pump equipped with Rheodyne Model 7125 injector with a 20-μl loop and a Shimadzu SPD-6A variable-wavelength UV detector. The detector output was monitored using a Shimadzu Chromatopac C-R5A.

Chiralcel OC, OG, OD and OF columns (250 × 4.6 mm I.D.; 10 μm particle size) used were purchased from Daicel (Tokyo, Japan), and the ovomucoid-conjugated silica gel column, Ultron ES-OVM (150 × 4.6 mm I.D.; 5 μm particle size), was purchased from Shinwa Kako (Kyoto, Japan).

2.2. Chemicals and materials

Diltiazem hydrochloride, *dl-cis*-diltiazem and *dl-trans*-diltiazem were synthesized by Tanabe Seiyaku (Osaka, Japan) [17–19]. HPLC-grade hexane, *n*-propanol, 2-propanol, acetonitrile, methanol and ethanol were obtained from Katayama (Tokyo, Japan). All other chemicals were of analytical-reagent grade or higher quality.

2.3. Chromatographic conditions

Chiralcel column

Samples were dissolved in ethanol to 1 mg/ml and the injection volume was 2–10 μl. The column temperature was maintained in the range 10–40°C using water-jacket thermostatic control. Hexane–2-propanol or –*n*-propanol containing 0–1% (v/v) diethylamine was used as the eluent at a flow-rate of 1.0 ml/min, and UV detection was carried out at 254 nm.

Ultron ES-OVM column

Samples were dissolved in ethanol to 1 mg/ml and the injection volume was 2–10 μl. The column temperature was maintained in the range 10–40°C using water-jacket thermostatic control. The mobile phases consisted of phosphate buffers at different pHs and ionic strengths with ethanol as the organic modifier, and the flow-rate was 1.0 ml/min. UV detection was carried out at 254 nm.

2.4. Sample preparation

Twenty Herbesser tablets were powdered. An amount equivalent to 50 mg of diltiazem hydro-

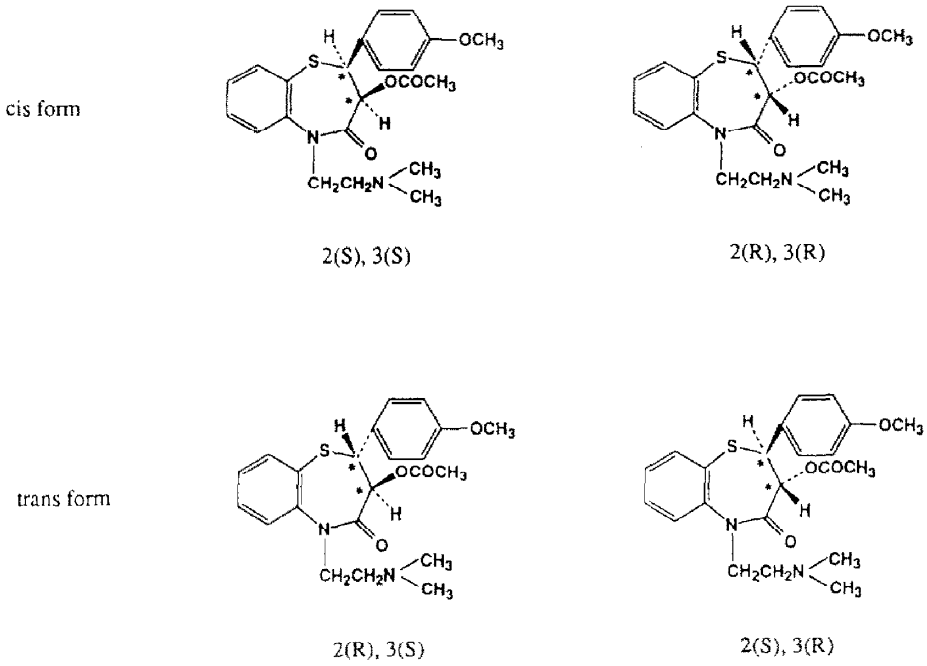

Fig. 1. Stereochemical structures of diltiazem.

chloride was weighed into a 100-ml volumetric flask, and 50 ml of ethanol were added. The flask was shaken for 15 min, ethanol was added to volume and the mixture was filtered. The filtrate was used as the sample solution (1 mg/ml of diltiazem hydrochloride) for injection into the HPLC system.

3. Results and discussion

The resolution of the four optical isomers of diltiazem hydrochloride shown in Fig. 1 was investigated by normal- and reversed-phase chiral HPLC using derivatized cellulose packings and an ovomucoid-conjugated column.

3.1. Normal-phase chiral HPLC

Different types of derivatized cellulose packings were investigated for the enantiomer separation of *cis*- and *trans*-diltiazem hydrochloride. The Chiralcel materials used (Fig. 2) are phenylcarbamate derivatives which are adsorbed on silica gel. Cellulose-based phases have mobile phase restrictions because the cellulose is soluble in certain solvents. Hexane with 2-propanol is generally used as the mobile phase and diethylamine is added (<1.0%) for basic compounds. Therefore, the enantiomer separation of *cis*- and *trans*-diltiazem hydrochloride was examined using as the mobile phase hexane–2-propanol containing diethylamine.

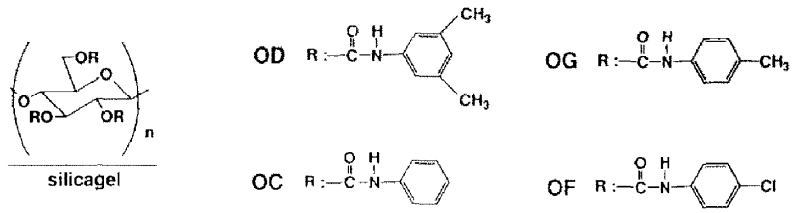

Fig. 2. Structures of Chiralcel packings.

Table 1
Effect of chiral stationary phase on separation of enantiomers of *cis*- and *trans*-diltiazem hydrochloride

Column (Chiralcel)	*dl-cis*-Diltiazem				*dl-trans*-Diltiazem			
	k'_1	k'_2	α	R_s	k'_1	k'_2	α	R_s
OC	5.34	9.05	1.69	1.76	5.25	(no separation)		
OG	3.13	4.43	1.42	2.49	2.75	3.17	1.15	0.99
OD	1.69	1.86	1.10	0.70	1.62	(no separation)		
OF	2.62	5.46	2.08	3.29	1.81	3.34	1.84	2.40

HPLC conditions: mobile phase, hexane–2-propanol (1:1, v/v) containing 1% (v/v) diethylamine; temperature, 30°C; flow-rate, 1 ml/min; detection, UV at 254 nm (0.32 AUFS). k'_1 = Capacity factor of first-eluting enantiomer; k'_2 = capacity factor of second-eluting enantiomer.

Table 1 summarizes the capacity factors (k'), separation factors (α) and resolutions (R_s) for the enantiomer separation of *cis*- and *trans*-diltiazem hydrochloride. These data show that the Chiralcel OF column gave the best resolution. In general, the separation of the enantiomers of *cis*-diltiazem hydrochloride was better than the separation of those of *trans*-diltiazem hydrochloride. In particular, the four optical isomers of diltiazem hydrochloride were completely resolved on the Chiralcel OF column (Fig. 3). In all instances, the *l*-isomers eluted first.

Okamoto *et al.* [20] showed the importance of hydrogen bonding between the carbamate group of the stationary phase and the solute for chiral recognition. As diltiazem hydrochloride has an ester group, such hydrogen bonding is likely to occur. This interaction might play an important role in chiral recognition. In addition, Okamoto *et al.* [20] also pointed out the participation of $\pi-\pi$ interactions of phenyl groups on the stationary phase with aromatic groups of the solute. The 2-phenyl ring of diltiazem hydrochloride could be involved in $\pi-\pi$ interactions. From the result, the interaction of *trans*-diltiazem with the chiral stationary phase, hydrogen bonding and $\pi-\pi$ interactions, etc., are assumed to be weaker than those for *cis*-diltiazem hydrochloride owing to steric hindrance. With cellulose trisphenylcarbamate derivatives, the substituent on the phenyl groups had a great influence on their optical resolving power. The substituent

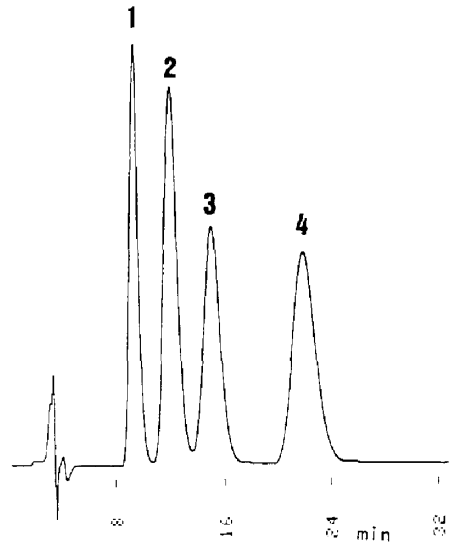

Fig. 3. Direct chromatographic separation of four optical isomers of diltiazem on Chiralcel OF. Peaks: 1 = *l-trans*-diltiazem; 2 = *l-cis*-diltiazem; 3 = *d-trans*-diltiazem; 4 = *d-cis*-diltiazem. Mobile phase: hexane–2-propanol (1:1, v/v) containing 0.1% (v/v) diethylamine; temperature, 30°C; flow-rate, 1 ml/min; detection, UV at 254 nm; sample loading, 10 μg.

appeared to change the ability of the carbamate moiety to undergo hydrogen bond formation [20].

Further analytical method development was investigated using the Chiralcel OF column. The effect of diethylamine was investigated in the concentration range 0–1.0%. Diethylamine concentration had little effect on retention. The resolution was constant and optimum with 0.05–0.5% of diethylamine. The values of α were 2.10–2.12 for *dl-cis* diltiazem hydrochloride and 1.95–1.97 for *dl-trans* diltiazem hydrochloride with 0.05–0.5% diethylamine. In order to prevent the detrimental effect of the basic mobile phase on the stationary phase, as ester groups are present in the packing material, only 0.1% (v/v) diethylamine was used in subsequent work.

Concentrations of 40–60% of 2-propanol in the mobile phase were investigated (Table 2), in the presence of 0.1% of diethylamine as a tailing-suppressing agent. An increase in 2-propanol concentration resulted in a corresponding decrease in retention. The resolution was im-

Table 2
Effect of 2-propanol concentration on k', α and R_s

Concentration of 2-propanol (%, v/v)	*dl-cis*-Diltiazem				*dl-trans*-Diltiazem			
	k'_1	k'_2	α	R_s	k'_1	k'_2	α	R_s
40	3.30	6.96	2.11	3.69	2.27	4.42	1.95	3.21
45	2.98	6.29	2.11	3.58	2.11	4.00	1.90	3.02
50	2.62	5.49	2.10	3.47	1.80	3.54	1.97	3.05
55	2.52	5.27	2.09	3.24	1.76	3.29	1.87	2.63
60	2.34	4.87	2.08	3.13	1.64	3.03	1.85	2.51

HPLC conditions: mobile phase, hexane–2-propanol containing 0.1% (v/v) diethylamine; temperature, 30°C; flow-rate, 1 ml/min; detection, UV at 254 nm (0.32 AUFS). k'_1 = Capacity factor of first-eluting enantiomer; k'_2 = capacity factor of second-eluting enantiomer.

proved with a decrease in 2-propanol concentration. The effect of the structure of the polar modifier was investigated using mobile phases of hexane with 2-propanol or *n*-propanol. *n*-Propanol decreased k', but the effect on α and R_s was not significant. Considering the enantiomeric separation, mutual selectivity and retention of *cis*- and *trans*-diltiazem hydrochloride, 50% of 2-propanol was selected as a polar modifier.

The chromatography was evaluated at temperatures from 20 to 40°C. The retention was lengthened when the column temperature was decreased. However, the effect on resolution was not significant.

3.2. Reversed-phase chiral HPLC

The enantiomer separation of *cis*- and *trans*-diltiazem hydrochloride was investigated on an Ulton ES-OVM column by changing mobile phase conditions such as modifier, pH, buffer concentration and temperature.

The effect of mobile phase was studied in the pH range 3.0–6.0 while maintaining a 20 m*M* phosphate buffer concentration in a buffer–ethanol (9:1, v/v) composition. The results are shown in Fig. 4 and indicate that resolution of the enantiomers of *cis*- and *trans*-diltiazem hydrochloride increased with increasing pH. *d-cis*-Diltiazem hydrochloride was retained strongly by the Ultron ES-OVM column at pH 6. Baseline separation between the enantiomers of *cis*-diltiazem hydrochloride was achieved throughout the pH range 4–6. However, the resolution of diastereomeric *l-cis*-diltiazem hydrochloride and *d-trans*-diltiazem hydrochloride was poor, with partial overlapping when the four optical isomers were chromatographed together.

The separation of the four optical isomers of diltiazem was investigated by changing the modifier (to acetonitrile or methanol), buffer concentration and temperature of column. The resolution of the enantiomers of *trans*-diltiazem hydrochloride was poorer using acetonitrile as the modifier. The resolution of diastereomeric *l-cis*-diltiazem hydrochloride and *d-trans*-diltiazem hydrochloride was poor with either

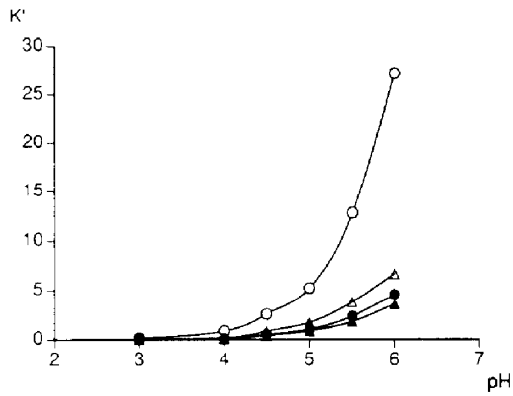

Fig. 4. Effect of pH on resolution of enantiomers of *cis*- and *trans*-diltiazem: ○ = *d-cis*-diltiazem; ● = *l-cis*-diltiazem; △ = *d-trans*-diltiazem; ▲ = *l-trans*-diltiazem. Column, ES-OVM; temperature, ambient; mobile phase, 20 m*M* phosphate buffer–ethanol (9:1); flow-rate, 1 ml/min; detection, UV at 254 nm; ambient. Sample loading, 2 μg.

methanol or ethanol as the modifier. As expected, the resolution was slightly improved by changing the ionic strength and column temperature. However, these effects were not very large and not important for chiral recognition.

The simultaneous resolution of four optical isomers of diltiazem hydrochloride was not achieved on the Ultron ES-OVM column. In general, the separation of the enantiomers of *cis*-diltiazem hydrochloride was better than that of the enantiomers of *trans*-diltiazem hydrochloride as on the Chiralcel column. In all instances the *l*-isomers eluted first as on the Chiralcel column.

3.3. Determination of three optical isomers in diltiazem hydrochloride

From the above evaluations, the optimum chromatographic conditions for the determination of three optical isomers in diltiazem hydrochloride using Chiralcel OF were defined as follows: column, Chiralcel OF; temperature, 30°C; mobile phase , hexane–2-propanol (1:1, v/v) containing 0.1% (v/v) diethylamine; and flow-rate, 1.0 ml/min. Typically, UV detection at 254 nm and a sample loading of 10 μg were used.

This chiral separation method was applied to the determination of optical impurity in the diltiazem hydrochloride product at low levels.

The method was examined for precision, accuracy, linearity and limit of detection under the above conditions. The precision of the method was evaluated by performing six replicate injections of a solution of *d-cis*-diltiazem hydrochloride containing ca. 1% of *l-cis*-, *d-trans*- and *l-trans*-diltiazem hydrochloride. The mean results were 0.95, 0.81 and 0.82% for *l-cis*-, *d-trans*- and *l-trans*-diltiazem hydrochloride, respectively, and the relative standard deviation was not more than 0.2%. The method was sufficiently precise.

The linearity and accuracy of the method were evaluated by recovery tests. The linearity and accuracy of method were determined by analysing spiked solutions containing 0.05, 0.1, 0.3, 0.5, 1.0 and 2.0% of *l-cis*-, *d-trans*- and *l-trans*-diltiazem hydrochloride in the presence of diltiazem hydrochloride. The method exhibited good linearity over the range tested, following

Table 3
Relationship between theoretical and found percentages

Optical impurity	Theoretical (%)	Found (%)	Difference (%)
l-cis-Diltiazem	0.05	0.07	+0.02
	0.10	0.12	+0.02
	0.30	0.31	+0.01
	0.49	0.48	−0.01
	0.96	0.99	+0.03
	1.85	1.86	+0.01
l-trans-Diltiazem	0.05	0.06	+0.01
	0.10	0.11	+0.01
	0.30	0.27	−0.03
	0.49	0.42	−0.07
	0.96	0.82	−0.14
	1.85	1.58	−0.28
d-trans-Diltiazem	0.10	0.10	0
	0.30	0.27	−0.03
	0.49	0.42	−0.07
	0.96	0.84	−0.12
	1.85	1.60	−0.26

HPLC conditions as in Fig. 3.

the linear equations $y = 0.9989x + 0.0148$ for *l-cis*-diltiazem hydrochloride, $y = 0.8593x + 0.0047$ for *d-trans*-diltiazem hydrochloride and $y = 0.8408x + 0.0153$ for *l-trans*-diltiazem hydrochloride, where y = observed response and x = theoretical response. The correlation coefficient was 1.00 for the three optical isomers. Therefore, the method was considered to be linear in the examined range of concentrations. The relationship between the theoretical and found percentages of each optical isomer is shown in Table 3. Good accuracy was obtained. The limit of quantification was determined to be about 0.05% for the three optical impurities in the presence of diltiazem hydrochloride, and the limit of detection was about 0.01% (1 ng) (signal-to-noise ratio = 4). Typical chromatograms of diltiazem hydrochloride spiked with ca. 0.05 and 0.1% of the three optical isomers are shown in Fig. 5.

The determination of optical impurities in diltiazem hydrochloride by the peak area percentage method showed acceptable precision, accuracy, linearity and detection limit.

Ten samples of diltiazem hydrochloride bulk drug and its tablets manufactured in Tanabe were analysed by this method. The results confirmed that the levels of optical impurities in diltiazem hydrochloride and tablets were below the detection limit (0.01%). The optical purity was excellent.

Finally, this method was compared with the methods reported previously. Enantiomer of diltiazem hydrochloride was first resolved by an indirect diastereomeric method using a chiral reagent followed by separation on a non-chiral column [9,10]. The indirect methods have several disadvantages, e.g., the cost and steady supply of the chiral reagent and the direct influence of

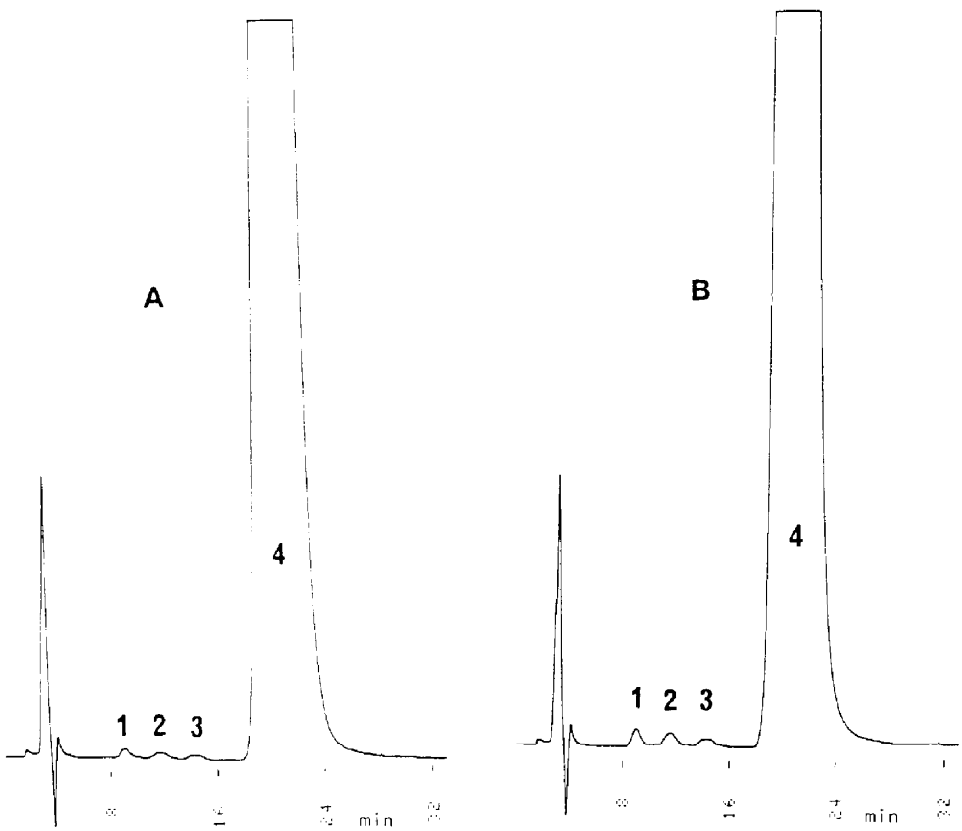

Fig. 5. Typical chromatograms of diltiazem spiked with (A) ca. 0.05 and (B) 0.1% of three optical isomers. Peaks: 1 = *l-trans*-diltiazem; 2 = *l-cis*-diltiazem; 3 = *d-trans*-diltiazem; 4 = *d-cis*-diltiazem. HPLC conditions as in Fig. 3.

the optical purity of the reagent on the analytical value. A method for the direct determination of the optical purity of diltiazem hydrochloride using an ES-OVM column has been reported [16]. However, the simultaneous determination of the three optical impurities in diltiazem hydrochloride was not achieved by the direct method on an ES-OVM column. The present method permitted the simultaneous determination of the three optical impurities in diltiazem hydrochloride bulk drug and those in tablets at levels dowm to ca. 0.05% by the area percentage method. Therefore, this method has advantages over our previous methods with regard to separation, sensitivity and ease of use.

4. Conclusions

Four optical isomers of diltiazem hydrochloride were successfully separated on a Chiralcel OF column in the normal-phase HPLC mode. The simultaneous determination of three optical impurities in diltiazem hydrochloride bulk drug and its tablets was successfully achieved at levels down to 0.05% by the area percentage method. The present method offers advantages over the reported methods, viz., a derivatization method and a direct method on an ES-OVM column, with regard to separation, sensitivity and ease of use. This method can be used for the investigation of the optical impurity profile of diltiazem hydrochloride in quality control and for the optimization of the optical resolution in the synthetic procedure, because the absolute configuration of optical impurities in diltiazem hydrochloride can be assigned by comparison with a reference sample. The method is also applicable to stereo-selective pharmacokinetic studies of diltiazem hydrochloride.

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