Journal of Chromatography A, 659 (1994) 467–471
Elsevier Science B.V., Amsterdam

CHROM. 25 693

Short Communication

Enantiomer separation of dihydropyridine derivative calcium antagonists by high-performance liquid chromatography with chiral stationary phases

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(First received June 7th, 1993; revised manuscript received October 28th, 1993)

ABSTRACT

The separation of enantiomers of dihydropyridine derivatives by high-performance liquid chromatography was studied using modified Pirkle-type chiral stationary phases. Resolution was achieved by normal-phase operation utilizing *n*-hexane–1,2-dichloroethane–methanol–trifluoroacetic acid as the mobile phase on two types of urea-derivative chiral stationary phase. Nine urea-type chiral phases were examined, derived from (*R*)-1-(α-naphthyl)ethylamine with (*S*)-proline or from (*S*)-1-(α-naphthyl)ethylamine with (*S*)-*tert.*-leucine chemically bonded to a 3-aminopropylsilanized silica stationary phase (Sumichiral OA-4500 and Sumichiral OA-4600).

INTRODUCTION

In recent years, the number of chiral dihydropyridine derivatives with calcium antagonist activity has rapidly increased. Dihydropyridine antagonists, as a racemic mixture of (+)- and (−)-dihydropyridine calcium antagonists, are undergoing clinical evaluation for antihypertensive and antianginal effects. For many of these derivatives, the individual enantiomers also have widely different biological activities [1,2]. It is important, therefore, to be able to determine the amount of each enantiomer. High-performance liquid chromatography (HPLC) on a chiral stationary phase is a very useful technique for the analysis of these enantiomers. Tokuma *et al.* [2] applied such a technique to the direct separation of nilvadipine enantiomers on a Chiralpak OT(+) analytical column, which is a (+)-poly-(triphenylmethyl methacrylate) stationary phase. It has also been reported that a stationary phase composed of a α_1-acid glycoprotein is able to separate a series of dihydropyridine enantiomers using a buffer [3]. Okamoto *et al.* [4] reported that optical resolution of dihydropyridine derivatives was possible on HPLC columns packed with xylan bis(3,5-dichlorophenylcarbamate) and cellulose tris(4-*tert.*-butylphenyl carbamate). However, the separation of enantiomers of recently developed chiral dihydropyridine derivatives could not be achieved by high-performance liquid chromatography on Pirkle-type chiral stationary phases. Oi and co-workers [5–8] previously reported the preparation of modified Pirkle-type columns and the enantiomeric sepa-

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SSDI 0021-9673(93)E1161-R

ration of chiral compounds using these chiral columns. These columns are useful for the separation of enantiomers of several biologically active compounds [6–8]. However, the direct resolution of enantiomers of dihydropyridine derivatives has not been reported using the above type of columns. In a previous paper [9], we described the enantiomer separation of dihydropyridine derivatives by liquid chromatography with a silica-based cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC) chiral stationary phase. However, this method was not adequate owing to the long analysis time and peak broading. In this paper, we describe the resolution, with sharp peaks, of the enantiomers of dihydropyridine derivatives by normal-phase HPLC on two types of chiral stationary phases. We examined nine urea-type chiral phases (Sumichiral OA-2000, OA-2500R, OA-3100, OA-4400, OA-4500, OA-4600, OA-4700, OA-4800 and OA-4900 in Fig. 2), derived from (*R*)-1-(α-naphthyl)ethylamine with (*S*)-proline or from (*S*)-1-(α-naphthyl)ethylamine with (*S*)-*tert.*-leucine chemically bonded to 3-aminopropylsilanized silica stationary phase (Sumichiral OA-4500 and Sumichiral OA-4600, respectively). *n*-Hexane–1,2-dichloroethane–methanol–trifluoroacetic acid was used as the mobile phase.

EXPERIMENTAL

Materials

Nilvadipine, nitrendipine, nisoldipine, nicardipine and benidipine (Fig. 1) were kindly donated by Fujisawa Pharmaceutical (Osaka, Japan), Yoshitomi Pharmaceutical (Osaka, Japan), Bayer (Wuppertal, Germany), Yamanouchi Pharmaceutical (Tokyo, Japan) and Kyowa Hakko Kogyo (Tokyo, Japan), respectively. *n*-Hexane and methanol were both of HPLC grade, and all other solvents and reagents were of analytical-reagent grade. Standard solutions of each dihydropyridine derivative were prepared in methanol and stored at 4°C, and 200–1000 ng of dihydropyridine derivatives were injected to the HPLC system.

Apparatus

The apparatus used for HPLC was a Waters Model 600 E high-performance liquid chromatograph equipped with a variable-wavelength UV detector (operated at 254 nm) (Millipore–Waters, Milford, MA, USA). The HPLC column contained Sumichiral OA-2000, OA-2500R, OA-3100, OA-4400, OA-4500, OA-4600, OA-4700, OA-4800 and OA-4900 chiral stationary phases

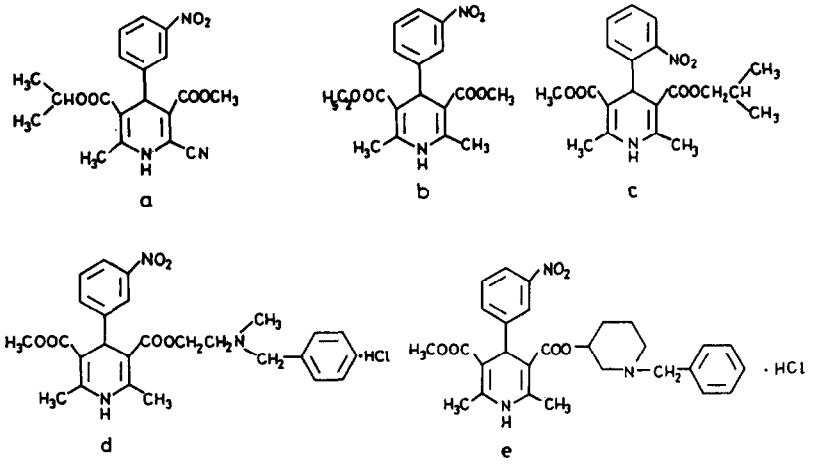

Fig. 1. Structures of dihydropyridine derivative calcium antagonists. (a) Nilvadipine; (b) nitrendipine; (c) nisoldipine; (d) nicardipine; (e) benidipine.

(Fig. 2) (5 or 15 μm, 250 × 4.6 mm I.D.) (Sumika Chemical Analysis Service, Osaka Japan). *n*-Hexane–1,2-dichloroethane–methanol-trifluoroacetic acid was used as the mobile phase.

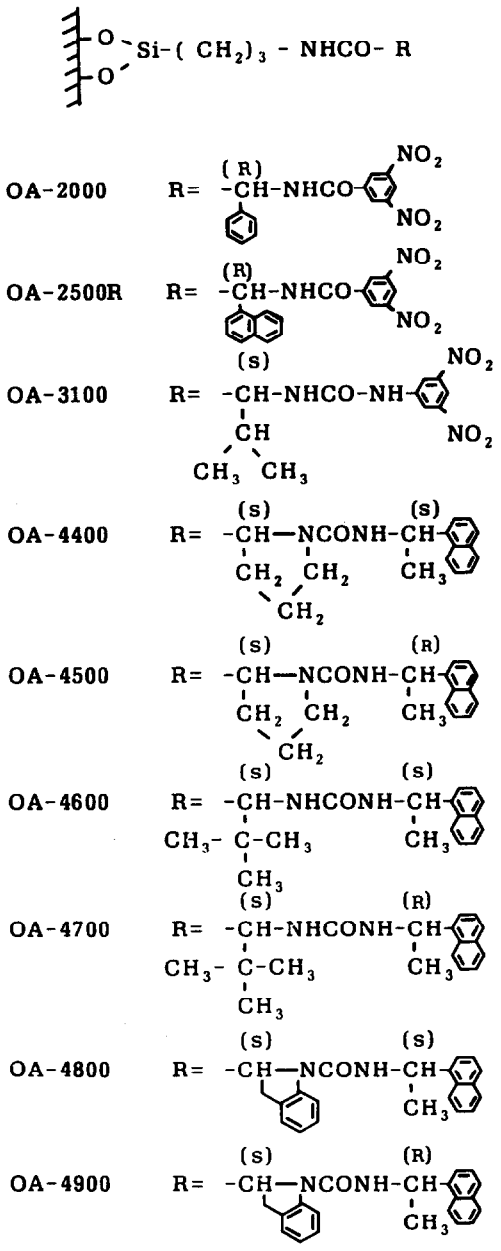

Fig. 2. Structures of chiral stationary phases. (Sumichiral OA).

Flow-rates of 1.0 ml/min were used at room temperature.

RESULTS AND DISCUSSION

We tried various chiral stationary and mobile phase systems for the enantiomeric separation of dihydropyridine derivatives (Fig. 1). The chromatographic results are summarized in Table I. The best separation was obtained for the enantiomers of nilvadipine and benidipine on the various chiral stationary phases used.

Effective enantioseparation of nilvadipine was obtained on Sumichiral-OA 4400 ($\alpha = 1.21$), 4500 ($\alpha = 1.39$), 4700 ($\alpha = 1.15$) and 4800 ($\alpha = 1.29$) using *n*-hexane–1,2-dichloroethane–methanol–trifluoroacetic acid (250:140:2:1) as the mobile phase. A typical chromatogram on a Sumichiral-OA 4500 column is shown in Fig. 3. Under the chromatographic conditions a resolution (R_s) of 3.6 was achieved for the nilvadipine enantiomers, with theoretical plate numbers of 2304 and 3927 for the (+) and (−)-enantiomers, respectively. We previously described the enantiomeric separation of nilvadipine on the silica-based cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC) (Chiralcel OD) [9]. However, this chromatographic system was not adequate owing to peak broadening and the long analysis time. In this study, we achieved a better chiral separation with a short analytical time with a Pirkle-type column rather than a CDMPC chiral column.

The separation of enantiomers of benidipine was obtained on Sumichiral OA-4500 ($\alpha = 1.30$), OA-4700 ($\alpha = 1.16$) and OA-4800 ($\alpha = 1.25$) using *n*-hexane–1,2-dichloroethane–methanol–trifluoroacetic acid (250:140:20:1) as the mobile phase, (Table I). A typical chromatogram is shown in Fig. 3. The resolution was $R_s = 4.0$ with theoretical plate numbers of 3410 for the (+)- and 4673 for the (−)-enantiomer.

The nisoldipine enantiomers were completely separated on Sumichiral OA-4600 only ($\alpha = 1.12$). A chromatogram of the enantiomeric separation of nisoldipine is shown in Fig. 3. The resolution was $R_s = 1.4$, with theoretical plate numbers of 2621 for the (+)- and for the (−)-

TABLE I

HPLC SEPARATION OF ENANTIOMERS OF DIHYDROPYRIDINE DERIVATIVES ON CHIRAL STATIONARY PHASES

Sumichiral	Nilvadipine			Benidipine			Nisoldipine			Nicardipine			Nitrendipine		
	M[a]	k_1	α	M[a]	k_1	α	M[a]	k_1	α	M[a]	k_1	α	M[a]	k_1	α
OA-2000	A	10.74	1.03	–	–	–	A	5.66	1.00	–	–	–	A	4.59	1.00
OA-2500R	–	–	–	D	6.57	1.04	–	–	–	D	6.82	1.00	–	–	–
OA-3100	–	–	–	D	27.29	1.07	–	–	–	D	24.17	1.00	–	–	–
OA-4400	A	6.77	1.21	D	3.52	1.00	A	2.77	1.00	D	2.91	1.00	A	2.72	1.00
OA-4500	A	4.67	1.39	E	12.40	1.30	A	1.49	1.00	E	8.30	1.05	C	5.17	1.00
OA-4600	A	5.96	1.10	D	2.45	1.11	B	4.17	1.12	D	3.04	1.00	B	5.87	1.00
OA-4700	A	2.25	1.15	D	1.59	1.16	A	1.23	1.00	D	1.47	1.00	A	1.05	1.00
OA-4800	A	4.82	1.29	D	4.73	1.25	A	2.80	1.04	D	4.71	1.00	A	1.92	1.00
OA-4900	A	13.43	1.04	D	4.42	1.00	A	4.21	1.08	D	4.12	1.00	A	3.12	1.00

[a] M = mobile phase: *n*-hexane–1,2-dichloroethane–methanol–trifluoroacetic acid, (A) 250:140:2:1, (B) 250:140:0.5:1, (C) 400:100:2:1, (D) 250:140:20:1 and (E) 250:140:10:1.

enantiomers 3226. Thus enantiomeric separation of nisoldipine could be obtained on the Pirkle-type column, but not on a CDMPC chiral column [9].

The enantiomers of nicardipine were almost separated on Sumichiral OA-4500 ($\alpha = 1.05$). The chromatogram is shown in Fig. 4. The resolution was $R_s = 1.1$, when theoretical plate numbers of 6297 for the (+)- and 6601 for the (−)-enantiomer. On the other hand, no separation of nitrendipine was obtained on a Pirkle-type column in these studies (Fig. 4). The separation factor of enantiomer for nitrendipine was 1.00 on the Sumichiral OA column (Table I), with a theoretical plate number of 1685.

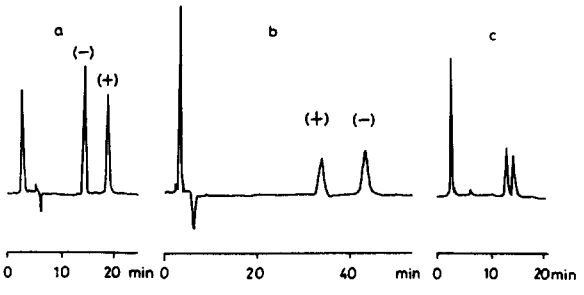

Fig. 3. Separation of dihydropyridine derivative calcium antagonists with chiral stationary phases (Sumichiral OA). Chromatographic conditions as in Table I. (a) Nilvadipine (OA-4500); (b) benidipine (OA-4500); (c) nisoldipine (OA-4600).

Delee *et al.* [3] reported the enantiomer separation of dihydropyridines on an α_1-acid glycoprotein column [3]. However, this method was not adequate owing to the long analytical time and peak broadening. The separation mechanism includes non-specific interaction of dihydropyridine with the α_1-acid glycoprotein stationary phase.

Okamoto *et al.* [4] also reported that a stationary phase composed of xylan bis(3,5-dichlorophenylcarbamate) and cellulose tris(4-*tert.*-butylphenylcarbamate) was able to separate a series of dihydropyridine enantiomers using a normal mobile phase [4]. The same group [10]

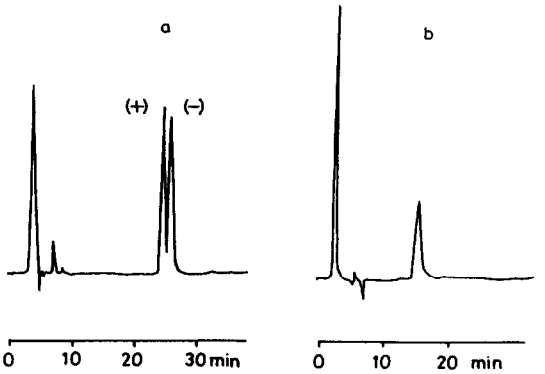

Fig. 4. Separation of dihydropyridine derivative calcium antagonists with chiral stationary phases (Sumichiral OA). Chromatographic conditions as in Table I. (a) Nicardipine (OA-4500); (b) nitrendipine (OA-4500).

also reported that the enantioseparation mechanism of polysaccharide phenylcarbamate-type stationary phases is considered to involve interactions between chiral adsorbing sites and the polar carbamate group. The groups can interact with a solute via hydrogen bonding with NH and C = O groups and dipole–dipole interactions on C = O. The adsorbing powers of these sites may be strongly influenced by the nature of the substituents on the phenyl group. The enantioseparation of dihydropyridine derivatives should be considered to follow the same mechanism on the polysaccharide phenylcarbamate stationary phase. On the other hand, Pirkle and Hamper [11] described the mechanism on a Pirkle-type column. The interaction mechanism with the chiral stationary phase was considered to require the analyte to contain a π-donor site, a basic site for association with the benzamide hydrogen and an acidic site to hydrogen bond to the basic carbonyl oxygen, and these sites must be stereochemically disposed such that all of the interactions with the chiral stationary phase can occur simultaneously.

From the results of this study, we consider that the enantioseparation of dihydropyridine derivatives involves interactions with the nitrobenzene ring, carbonyl group and amido group in the dihydropyridine molecule using Sumichiral OA stationary phases. Effective enantioseparation of nilvadipine was obtained because it contains a asymmetric dihydropyridine ring form 2-cyano group and a 5-carboxyisopropyl ester group. Adequate enantioseparation of benidipine was obtained. It is considered that the steric hindrance of the 1-benzyl-3-piperidyl moiety on the asymmetric carboxy group affected the interaction of benidipine on the chiral stationary phase. It was also considered that the steric hindrance of the isobutylmethyl moiety on the asymmetric carboxyl group affected the enantioseparation of nisoldipine on the chiral stationary phase. A poor enantioseparation of nicardipine was obtained. It is considered that the steric hindrance of the N-benzyl-N-methylaminoethyl moiety on the asymmetric carboxyl group weakly affected the interaction of nicardipine on the chiral stationary phase, because the bulky benzyl group is distant from the chiral centre. No enantioseparation of nitrendipine was obtained, because there is no steric hindrance of the ethyl moiety on the asymmetric carboxyl group. In conclusion, we found that Pirkle-type chiral stationary phases (Sumichiral OA) were very efficient for the separation of enantiomers of a few dihydropyridine calcium antagonists. We consider that the method using HPLC with these chiral stationary phases is very useful for the analysis of enantiomers of dihydropyridine calcium antagonists in clinical patients. Further applications of the method to enantiospecific therapeutic drug monitoring of dihydropyridine derivatives are being conducted.

ACKNOWLEDGEMENTS

The authors thank Dr. N. Oi and Dr. Y. Matsumoto of Sumika Chemical Analysis Service for helpful suggestions.

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