Journal of Chromatography, 592 (1992) 291–296
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2386

Direct separation of enantiomers by high-performance liquid chromatography on a new chiral ligand-exchange phase

Naobumi Ôi*, Hajimu Kitahara and Reiko Kira

Sumika Chemical Analysis Service, Ltd., 3-1-135, Kasugade-naka, Konohana-ku, Osaka 554 (Japan)

ABSTRACT

Two novel chiral ligand-exchange phases for high-performance liquid chromatography, N,S-dioctyl-(D)-penicillamine (I) and N,S-dioctyl-N-methyl-(D)-penicillamine (II) were prepared. The direct separation of more than twenty amino acid enantiomers was achieved using octadecylsilanized silica coated with these phases and water or hydro-organic eluents containing copper(II) ion as a mobile phase. Various enantiomers including amino acid derivatives, hydroxy acids and amino alcohols were also well resolved directly. Generally, phase I showed higher enantioselectivity than phase II, which, however, was very efficient for the separation of some long-retained racemic compounds such as tryptophan and mandelic acid. These phases are very promising for the direct separation of a variety of enantiomers by ligand-exchange high-performance liquid chromatography.

INTRODUCTION

Since the pioneering work of Davankov and co-workers [1] a great number of publications have appeared dealing with the separation of amino acid enantiomers by means of ligand-exchange high-performance liquid chromatography (HPLC) using either sorbents with covalently bonded chiral complex-forming ligands [1–6] or soluble chiral reagents in the mobile phase [7–9].

Davankov *et al.* [10] have presented a novel chiral-phase system which is composed of a reversed-phase packing coated with an appropriate resolving agent and a hydro-organic eluent containing the complexing metal ion. This system combines important advantages of the two approaches to resolving racemates by means of ligand-exchange chromatography: the use of chiral stationary phases and the use of chiral eluents. Many chiral coating agents, such as N-alkylhydroxyproline [10] (where alkyl is n-C_7H_{15}, n-$C_{10}H_{21}$ and n-$C_{16}H_{33}$), N-decylhistidine [11] and N,N-dioctylalanine [12], produced good enantioselectivity, but the separation of some amino acids was still not good enough.

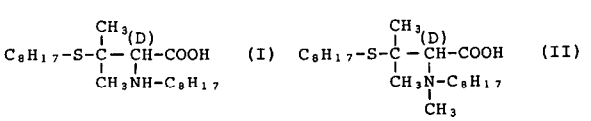

Fig. 1. Structures of phases I and II.

In this study two novel chiral ligand-exchange phases, N,S-dioctyl-(D)-penicillamine (I) and N,S-dioctyl-N-methyl-(D)-penicillamine (II) (Fig. 1), were prepared and their chromatographic properties were examined in order to investigate the effect of structure in the chiral ligand phase on the enantiomeric separation by ligand-exchange HPLC.

EXPERIMENTAL

Preparation of chiral stationary phases

N,S-dioctyl-(D)-penicillamine (phase I). This phase was prepared from (D)-penicillamine by the action of 1-bromooctane and triethylamine in a chloroform–methanol mixture at 70–80°C; m.p. 123–124°C. Analysis. Calculated for for $C_{21}H_{43}NO_2S$: C, 67.51; H, 11.60; N, 3.74%. Found: C, 67.12; H, 11.33; N, 3.68%. ^1H NMR (C^2HCl_3) δ 0.87 (t, 3H), 0.88 (t, 3H), 1.12–1.38 (m,

0021-9673/92/$05.00

20H), 1.41, 1.63 (s, 6H), 1.49–1.59 (m, 2H), 1.75–1.92 (m, 2H), 2.38-2.62 (m, 2H), 2.89–3.40 (m, 2H), 3.55 (s, 1H), 6.50 (m, 1H), 10.20 (s, 1H).

N,S-dioctyl-N-methyl-(D)-penicillamine (phase II). This phase wass prepared by the reductive N-methylation of N,S-dioctyl-(D)-penicillamine (I). Analysis. Calculated for $C_{22}H_{45}NO_2S$: C, 68.15; H, 11.72; N, 3.61%. Found: C, 68.05; H, 11.61; N, 3.68%. ^1H NMR (C^2HCl_3) δ 0.83 (t, 6H), 1.08–1.39 (m, 20H), 1.40–1.65 (m, 4H), 1.46, 1.47 (s, 6H), 2.40–2.61 (m, 2H), 2.71 (s, 3H), 2.82–3.29 (m, 2H), 3.51 (s, 1H), 10.20 (s, 1H).

General

Commercially available SUMIPAX ODS columns (150, 50 or 10 mm × 4 mm I.D.) packed with octadecylsilanized silica (5 μm) were used. The coating of phase I and II on the reversed-phase support was accomplished by passing a 0.1% methanol–water (50:50, v/v) solution of I and II through the column followed by a 1 m*M* aqueous solution of copper(II) sulfate. These columns are available from Sumika Chemical Analysis Service (Osaka, Japan), as Sumichiral OA-5000 and OA-5100. All chemicals and solvents of reagent grade were purchased from Wako (Osaka, Japan). The experiments were carried out using a Waters 510 high-performance liquid chromatograph equipped with a variable-wavelength UV detector (operated at 254 nm and 280 nm) or a fluorescence detector. In the fluoro-

TABLE I

ENANTIOMER SEPARATION OF AMINO ACIDS

Mobile phase: (A) 1 m*M* copper(II) sulfate in water; (B) 2 m*M* copper(II) sulfate in water–methanol (85:15); (C) 2 m*M* copper(II) sulfate in water–methanol (70:30). A flow-rate of 1 ml/min was typically used for the 150 mm × 4.6 mm I.D. column at room temperature. An injection volume of 5 μl (2 mg/ml) was typically used. k'_L, k'_D = capacity factors of L- and D-isomer; α = separation factor (k'_D/k'_L)

Amino acid	Phase I				Phase II			
	k'_L	k'_D	α	Mobile phase	k'_L	k'_D	α	Mobile phase
Ornithine	0.86	1.04	1.21	A	0.52	0.34	0.65	A
Lysine	1.06	1.61	1.52	A	1.06	1.06	1.00	A
Arginine	1.72	3.26	1.90	A	2.08	2.59	1.25	A
Serine	3.28	3.75	1.14	A	1.83	1.56	0.85	A
Alanine	3.49	5.52	1.58	A	2.95	3.66	1.24	A
Threonine	3.86	4.57	1.18	A	2.41	2.41	1.00	A
Glutamine	3.99	6.66	1.67	A	3.53	4.20	1.20	A
Asparagine	4.03	4.03	1.00	A	2.00	1.18	0.59	A
2-Amino-*n*-butyric acid	6.75	11.70	1.73	A	7.61	8.96	1.18	A
Proline	8.76	21.33	2.43	A	11.42	10.29	0.90	A
Valine	12.57	23.68	1.88	A	17.81	16.26	0.91	A
Norvaline	18.31	35.16	1.92	A	18.14	26.02	1.43	A
Histidine	23.11	17.55	0.76	A	7.42	5.20	0.70	A
tert.-Leucine	7.49	13.58	1.81	B	6.37	7.73	1.21	B
Alloisoleucine	8.18	13.29	1.62	B	5.08	4.66	0.92	B
Methionine	8.36	10.68	1.28	B	5.00	5.00	1.00	B
Isoleucine	9.98	16.38	1.64	B	5.08	6.71	1.32	B
Aspartic acid	10.87	15.27	1.40	B	1.02	0.87	0.85	B
Leucine	11.51	17.68	1.54	B	6.27	9.52	1.52	B
Glutamic acid	20.74	22.80	1.10	B	1.69	2.27	1.34	B
Phenylglycine	4.74	7.52	1.59	C	2.48	7.99	3.22	C
Tyrosine	5.20	6.79	1.31	C	3.21	2.85	0.89	C
DOPA[a]	5.69	7.25	1.27	C	3.69	3.69	1.00	C
Phenylalanine	11.51	16.19	1.41	C	10.16	8.66	0.85	C
Tryptophan	34.49	37.60	1.09	C	24.23	19.21	0.79	C

[a] 3,4-Dihydroxy-phenylalanine.

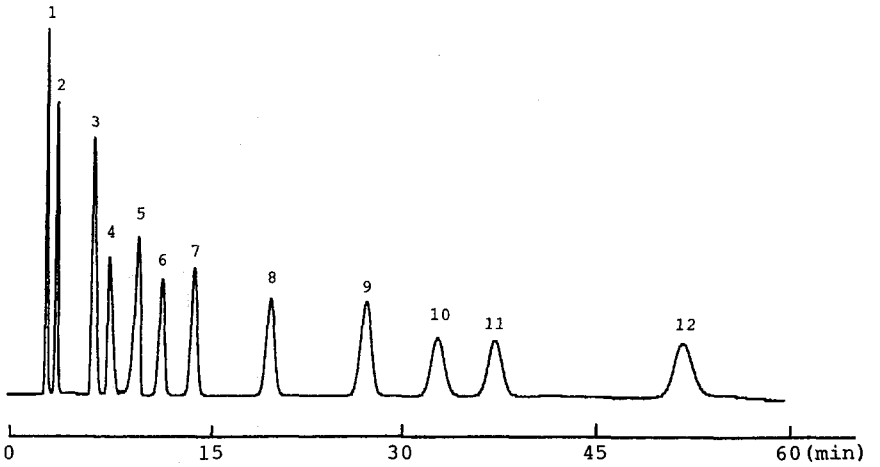

Fig. 2. Enantiomeric separation of six racemic amino acids on phase I. Chromatographic conditions as in Table I. Peaks: 1 = L-lysine; 2 = D-lysine; 3 = L-alanine; 4 = L-glutamine; 5 = D-alanine; 6 = D-glutamine; 7 = L-proline; 8 = L-valine; 9 = L-norvaline; 10 = D-proline; 11 = D-valine; 12 = D-norvaline.

metry, the post-column reaction using *o*-phthalaldehyde was employed [13].

RESULTS AND DISCUSSION

The direct separation of more than twenty racemic amino acids was accomplished by ligand-exchange HPLC using octadecylsilanized silica coated with phase I and II and aqueous or hydro-organic solutions containing copper(II) ion as a mobile phase. The capacity factors and separation factors are summarized in Table I. Excellent enantioselectivities were obtained with the two phases at room temperature. These results show that the structures of two phases are effective for the chiral recognition of a wide range of amino acids.

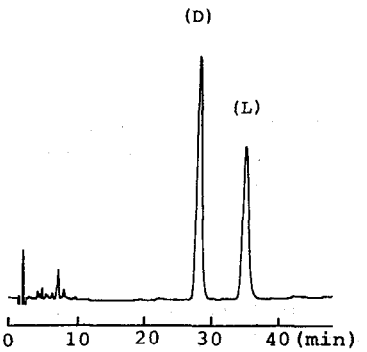

Fig. 3. Enantiomeric separation of racemic tryptophan on phase II. Chromatographic conditions as in Table I.

Generally, higher retention and enantioselectivity were obtained with the phase I system than with the pase II system. Fig. 2 shows a typical chromatogram of six amino acids on phase I. Larger separation factors and more moderate capacity factors were obtained for some amino acids, such as glutamic acid and tryptophan, on phase II than on phase I (Fig. 3). There was a very noticeable difference in the elution order of enantiomers between the phase I system and the phase II system. The D-isomer was always more strongly retained than the L-isomer on phase I, with the exception of histidine. The inverse elution order of the enantiomers of histidine may depend upon the nature of the imidazole group of histidine, as indicated by Davankov *et al.* [10]. On the other hand, the L-isomer was strongly retained in many amino acids on phase II. As both phases contain the same alkyl chain, these chromatographic results suggest that the hydrogen bonding effect of the NH group in phase I and the steric effect of the N-CH_3 group in phase II may produce a difference in the three-dimensional interaction for chiral recognition. We consider that the explanation of the recognition mechanism needs further examination.

Table II summarizes the results of the separation of racemic N-acetylamino acids, glycylpeptides and

TABLE II

ENANTIOMER SEPARATION OF N-ACETYLAMINO ACIDS, GLYCYL DIPEPTIDES AND HYDROXY ACIDS

Mobile phase: (A) 1 m*M* copper(II) sulfate in water; (B) 2 m*M* copper(II) sulfate in water–methanol (85:15); (C) 2 m*M* copper(II) sulfate in water–methanol (70:30). A flow-rate of 1 ml/min was used for the 150 mm × 4.6 mm I.D. column at room temperature. An injection volume of 5 μl (2 mg/ml) was typically used. k'_1, k'_2 = capacity factors of first- and second-eluted isomer; α = separation factor (k'_2/k'_1)

Compound	Phase I				Phase II			
	k'_1	k'_2	α	Mobile phase	k'_1	k'_2	α	Mobile phase
N-Acetylalanine	6.34	7.24	1.14	B	2.29	2.92	1.28	B
N-Acetylvaline	20.33	29.43	1.45	C	6.95	8.05	1.16	C
N-Acetylmethionine	21.10	25.97	1.23	C	8.66	10.00	1.15	C
N-Acetylleucine[a]	37.45	50.59	1.35	C	24.00	33.52	1.40	C
Glycylalanine	1.52	2.71	1.78	A	0.55	0.74	1.35	A
Alanylglycine	0.66	1.49	2.26	A	0.56	0.66	1.18	A
Glycylvaline	5.54	9.39	1.69	C	0.62	1.27	2.05	C
Alanylglycyl-glycine	2.56	4.15	1.62	A	1.21	1.52	1.26	A
Leucylglycyl-glucine	4.92	6.67	1.36	C	1.28	1.44	1.13	C
Lactic acid	14.66	19.32	1.32	A	8.77	9.94	1.13	A
Glyceric acid	10.73	14.41	1.34	B	2.29	2.85	1.24	B
2-Hydroxy-*n*-butyric acid	15.30	24.61	1.61	B	7.69	9.75	1.27	B
Leucic acid[a]	44.94	62.96	1.40	C	35.18	49.21	1.40	C
Mandelic acid[a]	42.64	54.73	1.28	C	24.59	61.31	2.49	C
Malic acid[b]	24.10	268.95	11.16	C	1.20	4.90	4.08	C
Tartaric acid[b]					9.85	15.95	1.62	C

[a] Column: 50 mm × 4.6 mm I.D.
[b] Column: 10 mm × 4.6 mm I.D.

hydroxy acids. These enantiomers could be well resolved in the same way as amino acids. Racemic hydroxy acids are generally more retained on phase I. Faster elution was achieved by the addition of methanol or acetonitrile, or by the use of shorter columns with both phases. An example of the efficient chromatogram is shown in Fig. 4.

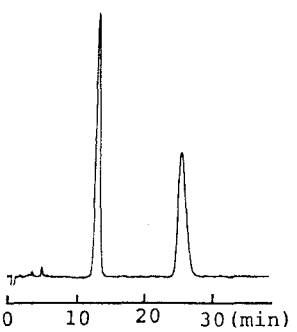

Fig. 4. Enantiomeric separation of racemic mandelic acid on phase II. Chromatographic conditions as in Table II.

Amino alcohol enantiomers of pharmaceutical interest were well resolved with these novel phases, as shown in Table III. The effect on these separations of the pH and the concentration of copper(II) ion in the eluent was large as reported by Yamazaki *et al.* [13], and good results were obtained with the eluent containing 5 m*M* copper(II) acetate at pH 6.0. Fig. 5 shows a typical chromatogram. The post-column derivatization with *o*-phthalaldehyde and subsequent fluorimetric detection was available for some amino alcohols, as shown in Fig. 6. Some other racemic compounds, for example 3-amino-ε-caprolactam, can be also resolved with these phases, as shown in Fig. 7.

As far as column durability is concerned, it can be stated that 600 analyses of some amino acids on the column coated with phase I did not cause any change in their retention parameters, enantioselec-

TABLE III

ENANTIOMER SEPARATION OF AMINO ALCOHOLS AND OTHERS

Mobile phase: (A) 5 m*M* copper(II) acetate in water (pH 6.0); (B) 0.05 *M* ammonium acetate buffer (pH 6.0) containing 5 m*M* copper-(II) acetate; (C) 1 m*M* copper(II) sulfate in water. A flow-rate of 1 ml/min was typically used for the 150 × 4.6 mm I.D. column at room temperature. An injection volume of 5 μl (2 mg/ml) was typically used. k'_1, k'_2 = capacity factors of first- and second-eluted isomer; α = separation factor (k'_2/k'_1)

Compound	Phase I				Phase II			
	k'_1	k'_2	α	Mobile phase	k'_1	k'_2	α	Mobile phase
Octopamine	1.98	2.53	1.28	A	1.09	1.61	1.48	A
Norphenylephrine	6.61	8.41	1.27	A	3.06	4.58	1.50	A
Normethanephrine	5.47	6.73	1.23	A	2.57	3.94	1.53	A
p-Hydroxynorephedrine	2.07	2.95	1.43	A	1.71	2.18	1.27	A
Norephedrine[a]	15.28	28.14	1.84	B	16.60	21.11	1.27	B
2-Amino-1-phenylethanol[a]	14.14	19.57	1.38	B	10.42	12.95	1.24	B
3-Amino-ε-caprolactam	2.01	2.61	1.30	C	1.80	2.83	1.57	C
Homocystein thiolactone	1.46	1.66	1.14	C	1.18	1.18	1.00	C

[a] Detected with a fluorescence detector.

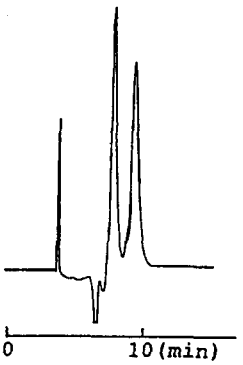

Fig. 5. Enantiomeric separation of racemic norphenylephrine on phase I. Chromatographic conditions as in Table III.

tivity or efficiency when water or methanol–water (30:70, v/v) was used as the eluent. Unfortunately however, the stability of the column coated with phase II was not as good as that of the phase I column, therefore the phase II system should be utilized carefully.

CONCLUSION

Two novel chiral phases, I and II, are very promising as coating agents on reversed-phase materials

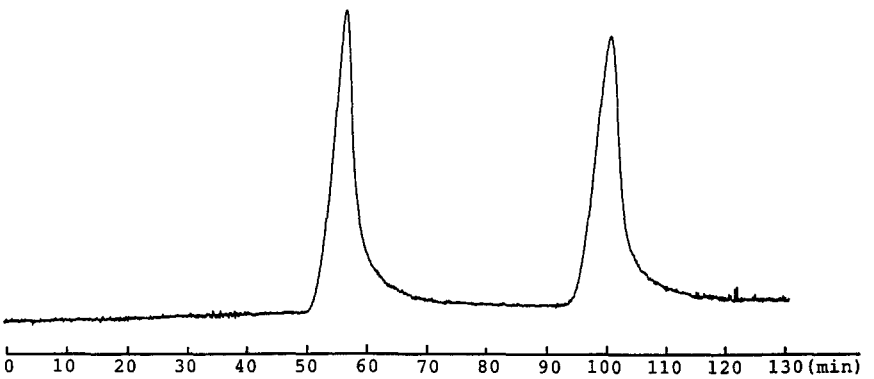

Fig. 6. Enantiomeric separation of racemic norephedrine on phase I. Chromatographic conditions as in Table III.

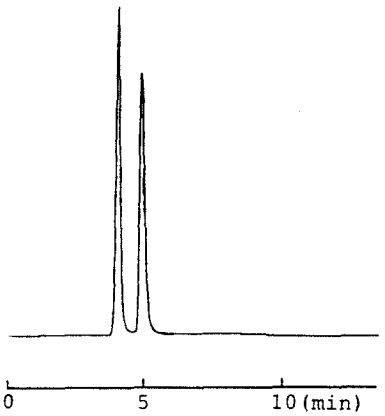

Fig. 7. Enantiomeric separation of racemic 3-amino-ε-caprolactam on phase I. Chromatographic conditions as in Table III.

for the direct separation of a variety of enantiomers including amino acids, hydroxy acids and amino alcohols by ligand-exchange HPLC.

ACKNOWLEDGEMENTS

We gratefully acknowledge the assistance of Miss F. Aoki, Mr. T. Harimoto and Mr. K. Kitasaka.

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