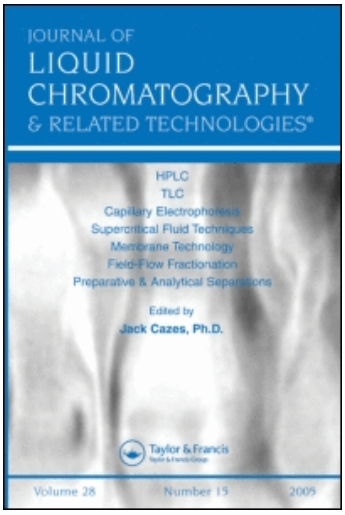

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RETENTION BEHAVIOR OF DERIVATIZED AMINO ACIDS AND DIPEPTIDES IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING CYCLODEXTRIN AS A MOBILE PHASE ADDITIVE

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ABSTRACT

The chromatographic behavior of amino acids and dipeptides derivatized with dansylchloride or (S)-1-(1-naphthyl)ethyl isocyanate was examined by reversed-phase high-performance liquid chromatography using cyclodextrin as the mobile phase additive.

The addition of β-cyclodextrin to the mobile phase was effective for the optical resolution of dansyl amino acids but not for that of dansyl dipeptides. The separation of diastereomeric dipeptides was slightly improved by the additive.

INTRODUCTION

Optical resolution has been achieved by high-performance liquid chromatography (HPLC) with pre-column derivatization with a chiral reagent, a chiral stationary phase or mobile phase

containing a chirality-recognizing reagent. Optical resolution by the addition of a chirality-recognizing reagent relies on chiral recognition by ligand exchange, ion-pair-formation and inclusion complexing [1]. It is well known that cyclodextrin (CD) forms inclusion complexes with a variety of molecules and ions. Enantiomeric resolution of dansyl (DNS) amino acids by micro HPLC using β-CD as the mobile phase additive was reported by Takeuchi et al. [2].

In previous papers we reported the much improved separation of steroids has been observed by the addition of the suitable CD to the mobile phase in reversed-phase HPLC [3]. The present paper deals with the chromatographic behavior of amino acids and peptides derivatized with DNS chloride or (S)-1-(1-naphthyl)ethyl isocyanate (NEI) in reversed-phase HPLC using CD as the mobile phase additive.

MATERIALS AND METHODS

Materials

α-, β- and γ-CDs were kindly supplied by Nihon Shokuhin Kako (Tokyo, Japan). Heptakis-(2,6-di-O-methyl)-β-CD (Me-β-CD; 10.5 methyl residues/mol) was prepared and donated by Kao (Tokyo). Amino acids, peptides, DNS chloride and NEI were obtained from Tokyo Kasei Kogyo (Tokyo). Baclofen was kindly donated by Ciba-Geigy (Basel, Switzerland). Derivatizations with DNS chloride

and NEI were done according to the reported methods [2, 4], respectively.

Apparatus

HPLC was carried out on a Shimadzu LC-6A chromatograph (Shimadzu, Kyoto, Japan) equipped with a Hitachi F-1050 fluorescence detector (Hitachi, Tokyo) at a flow-rate of 1.0 ml/min. The wave lengths of excitation (λex) and emission (λem) were set as follows: DNS derivative (λex 366 nm, λ em 510 nm), (S)-1-(1-naphthyl)ethyl carbamoyl (NEC) derivative (λex 235 nm, λem 333 nm). A TSKgel ODS-80TM (5 μm: 15 cm x 0.46 cm i.d.; TOSOH, Tokyo) and a YMC-GEL C_8 (5 μm: 15 cm x 0.4 cm i.d.; YMC, Kyoto) columns were used under ambient conditions, and the void volume was measured with methanol (λex 280 nm, λ em 320 nm). The pH of the mobile phase was adjusted with H_3PO_4.

RESULTS AND DISCUSSION

Effect of CD on the Retention of DNS Amino Acids

Takeuchi et al. reported the enantiomeric resolution of DNS amino acids by micro HPLC using β-CD as the mobile phase additive [2], but the effect of other CD has not been reported precisely. On the basis of these findings, the effect of α -, β -, Me-β- and γ-CD contents in the mobile phase on the relative capacity factots (Rk') of DNS-Gly, -L-Ala and -L-Thr using conventional reversed-phase column was investigated. It is necessary to use a

TABLE 1. Effect of CD on the Rk' Values of DNS Amino Acids

	t_R (min)*	Rk'**			
		α-CD	β-CD	Me-β-CD	γ-CD
Gly	16.1	0.94	0.88	0.58	0.83
L-Ala	19.8	0.93	0.89	0.53	0.83
L-Thr	17.0	0.92	0.88	0.51	0.84

* Conditions: column, YMC-GEL C_8; mobile phase, MeOH-0.5% K_2HPO_4 (pH 6.5)(35:65).

** The k' value obtained without CD was taken as 1.0. Each CD (2 mM) was added in the mobile phase.

mobile phase containing water in inclusion chromatography using CD as the mobile phase additive [5]. It is possible for a octyl silyl-coated (C_8) column to elute the derivative with a mobile phase containing a large proportion of water than octadecyl-coated (C_{18}) column. YMC-GEL C_8 column was used for this purpose and the results were shown in TABLE 1.

Among the CDs examined, α-CD showed the least effect on the k' values of the examined DNS amino acids. Not so difference has been observed on the effects of β- and γ-CDs. On the contrary, the k' values of these compounds were most influenced by Me-β-CD, but it took at least more than 200 ml for washing the column with the mobile phase containing Me-β-CD to get the constant value. These phenomena are more clearly observed by using C_{18} column. These data suggest the dynamic coating of the reversed-phase column has occurred with methylated CD as reported [6].

Enantiomeric Resolution of DNS-Amino Acids and -Dipeptides

The effect of each CD on the resolution of enantiomeric DNS Thr was examined and the results were summarized in TABLE 2. According to the previous report [2], the C_{18} column (TSKgel ODS-80TM) was chosen for this purpose. Although the addition of Me-β-CD gave the shortest t_R, the separation has not been observed. The mobile phase containing γ-CD did not elute the derivative under the examined conditions. The data showed that β-CD is most effective for this resolution, so the following enantiomeric resolution has been done with β-CD as shown in TABLE 3. Almost the same resolution as reported has been obtained by this conventional HPLC [2], but the separation of enantiomeric DNS-baclofen, -Gly-Leu and -Leu-Gly has not been done under several examined conditions using C_{18} and C_8 columns.

TABLE 2. Enantiomeric Resolution of DNS Thr

	t_R (min)			
	Me-β-CD*	β-CD*	β-CD**	γ-CD**
D-Thr	9.5	85.7	11.6	n.d.***
L-Thr	9.3	78.3	11.0	n.d.
Rs	0	2.2	1.2	-

Conditions: column, TSKgel ODS-80TM; mobile phase, MeCN-0.5% K_2HPO_4 (pH 6.1) [*(10:90), **(20:80)] containing 12.5 mM CD as indicated.
*** not detectable within 90 min.

TABLE 3. Enantiomeric Resolution of DNS Amino Acids

	Mobile phase*	t_R (min)		Rs
		D	L	
Ala	15:85	36.5	38.0	0.9
Leu	25:75	16.4	17.0	0.8
Phe	25:75	22.9	23.7	0.8
Ser	15:85	24.7	25.9	1.0

* Conditions: column, TSKgel ODS-80TM; mobile phase, MeCN-0.5% K_2HPO_4 (pH 6.5) containing 12.5 mM β-CD. The ratio was as indicated.

Effect of CD on the Separation of Diastereomeric Amino Acids and Peptides

Amino acids and peptides were derivatized with NEI according to the reported method [4] and the separation of the obtained diastereomeric naphthylethyl carbamoyl (NEC) derivatives was attempted as follows. The much improved separation of NEC amino acids than the reported one [4] has been obtained and summarized in TABLE 4. The satisfactory separation (Rs 1.3) of diastereomeric NEC Baclofen was also done and its chromatogram was shown in Fig. 1a. But the addition of CD was not so effective for the separation of these diastereomers.

The separation of diastereomeric NEC dipeptides was also examined and the results were summarized in TABLE 5. All the diastereomeric dipeptides were clearly separated without the addition of CD. Addition of 5 mM of γ-CD gave the slight

TABLE 4. Separation of Diastereomeric NEC Amino Acids

	Mobile phase*	t_R (min)		Rs
		D	L	
Ser	20:80	10.7	11.5	1.3 (1.0)**
Thr	23:77	6.8	8.0	2.4 (1.7)
Ala	23:77	8.1	9.1	1.8 (1.4)
Leu	25:75	14.5	19.0	5.6 (2.7)
Tyr	40:60	10.8	12.1	2.1 (0.9)

* Conditions: column, TSKgel ODS-80TM; mobile phase, MeCN-0.5% K_2HPO_4 (pH 6.5). The ratio was as indicated.
** Reported Rs value [4].

TABLE 5. Effect of CD on the Separation of Diastereomeric NEC Dipeptides

	Mobile phase*	without CD		γ-CD		Me-β-CD	
		t_R (min)	Rs	Rk'**	Rs	Rk'	Rs
Gly-D-Ser	20:80	14.6		0.95		0.58	
Gly-L-Ser		13.4	1.9	0.95	1.9	0.59	1.3
Gly-D-Thr	20:80	16.8		0.94		0.57	
Gly-L-Thr		15.5	1.8	0.93	1.9	0.57	1.4
Gly-D-Leu	25:75	18.8		1.00		0.80	
Gly-L-Leu		16.8	2.4	0.99	2.6	0.80	2.3
D-Leu-Gly	25:75	20.9		0.99		0.80	
L-Leu-Gly		29.4	7.6	1.01	8.2	0.79	7.1

* Conditions: column, TSKgel ODS-80TM; mobile phase, MeCN-0.5% K_2HPO_4 (pH 6.5) containing CD (0 or 5 mM) as indicated. The ratio was as indicated.
** The k' value obtained without CD was taken as 1.0.

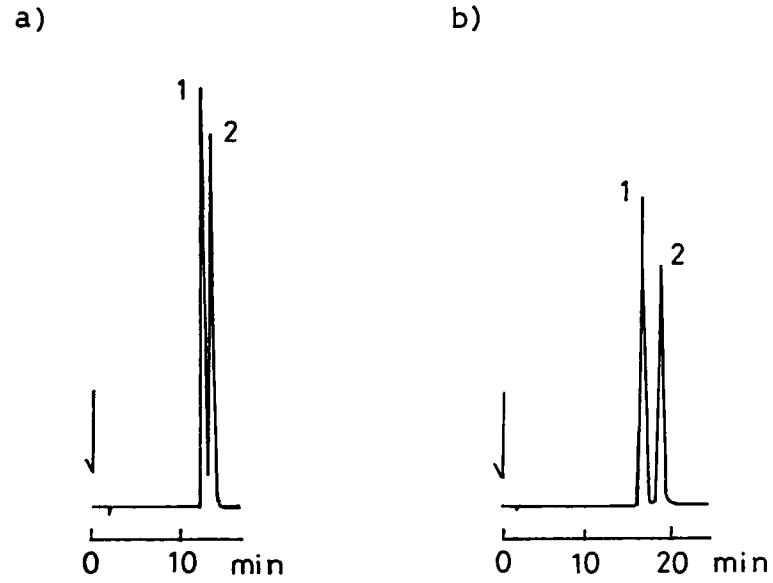

FIGURE 1. (a) Separation of Diastereomeric NEC Baclofen. Conditions: column, TSKgel ODS-80TM; mobile phase, MeOH-0.5% K_2HPO_4 (pH 6.5)(60:40).

1. NEC (R)-baclofen 2. NEC (S)-baclofen

(b) Separation of Diastereomeric NEC Gly-Leu. Conditions: column, TSKgel ODS-80TM; mobile phase, MeCN-0.5% K_2HPO_4 (pH 6.5)(25:75) containing 5 mM of γ-CD.

1. NEC Gly-L-Leu 2. NEC Gly-D-Leu

decrease of k' and increased Rs value (Fig. 1b). On the other hand addition of Me-β-CD gave the significant decrease of k' and slight decrease of Rs value.

CONCLUSION

The chromatographic behavior of DNS-amino acids, -dipeptides, NEC-amino acids and -dipeptides was examined with

reversed-phase HPLC using CD as a mobile phase additive. The addition of suitable CD to the mobile phase was effective for the optical resolution of DNS amino acids and slightly effective for the separation of diastereomeric dipeptides.

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