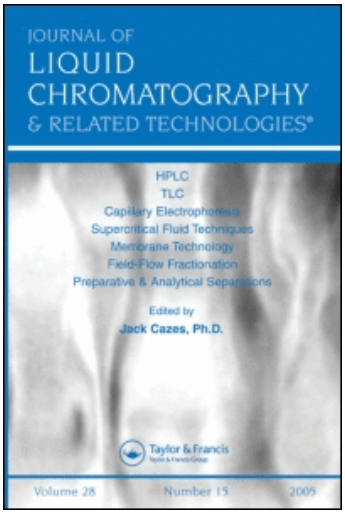

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Separation of Enantiomers Using an (S)-Naphthylethylcarbamoylated γ-Cyclodextrin Stationary Phase

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To cite this Article Nakatsu, Curtis N. and Stalcup, Apryll M.(1993) 'Separation of Enantiomers Using an (S)-Naphthylethylcarbamoylated γ-Cyclodextrin Stationary Phase', Journal of Liquid Chromatography & Related Technologies, 16: 1, 209 — 223
To link to this Article: DOI: 10.1080/10826079308020907
URL: http://dx.doi.org/10.1080/10826079308020907

JOURNAL OF LIQUID CHROMATOGRAPHY, 16(1), 209-223 (1993)

SEPARATION OF ENANTIOMERS USING AN (S)-NAPHTHYLETHYLCARBAMOYLATED γ-CYCLODEXTRIN STATIONARY PHASE

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ABSTRACT

Silica bonded γ-cyclodextrin was derivatized to contain approximately 9 (S)-naphthylethyl carbamate substituents per γ-cyclodextrin. The derivatized γ-cyclodextrin-based stationary phase was used to resolve a number of enantiomeric pairs using high performance liquid chromatography under normal phase conditions. Several 3,5-dinitrobenzoyl derivatized amino acids were successfully separated. In general, selectivities (α) achieved were greater than 1.1. The chiral recognition mechanism appears to be similar to an analogous derivatized ß-cyclodextrin based stationary phase. Possible explanations for the differences between the two are discussed.

INTRODUCTION

The efficacy of a drug within a biological system is dependent on how well it can bind to various receptors and/or enzymes. For the most part, efficient binding requires a three

dimensional fit. Hence, stereoisomers can have significantly different effects [1]. In some cases, one enantiomer may elicit the therapeutic effect while the other may be toxic. It is also possible for the isomers within racemic mixtures (50% of each isomer) to affect the actions of one another through competitive binding [2].

Currently, about 88% of all synthetic chiral drugs are marketed and produced as the racemic mixture [3]. This figure has raised some concern at the Food and Drug Administration causing them to consider stricter quality control specifications. Proposed guidelines would require the pharmaceutical industry to test the biotoxicity as well as the therapeutic effects of each isomer as well as the racemic mixtures of marketed drugs [4]. Similar conditions are anticipated for the food and beverage industry [5]. Such intensive testing would demand efficient methods for separating enantiomers. Fortunately, the chromatographic technology is fairly well developed to accomplish these separations with over 50 chiral stationary phases (CSPs) currently available for use with high performance liquid chromatography (HPLC) [6].

The possibility of using cyclodextrins (CDs) for separating enantiomeric mixtures was proposed as early as 1959 [7]. CDs are a group of macrocyclic molecules comprised of units of glucose. They feature a toroidal shape that embodies an apolar cavity and a polar exterior [8]. The polar exterior is the result of two groups of hydroxyls. Each glucose unit contributes one primary hydroxyl, which is positioned at the narrower base of the CD. In addition, each glucose unit provides two secondary hydroxyls positioned at the wider mouth of the CD [9,10]. Currently, there are three types of native CD that are available

commercially with the only difference between the three being the number of glucose units incorporated into the macrocyclic structure (e.g α-CD has 6; ß-CD has 7; and γ-CD has 8) [8].

The CD's separating capabilities have been studied quite extensively over recent years. Although native CD based stationary phases have been shown to be effective in separating many classes of compounds [11,12,13,14], their ability to separate enantiomers using HPLC is of particular interest.

Initial chromatographic experiments with the native CD based stationary phases were, for the most part, confined to reversed-phase conditions. Enantioselectivity of these phases was thought to be dependent upon hydrophobically-driven inclusion complexation of the solute with the native CD cavity [15,16]. Under normal phase conditions, the non-polar component of the mobile phase was thought to occupy the CD cavity thereby preventing the solute from forming an inclusion complex [17]. Recently, however, native CD phases have been used effectively for chiral separations with polar, organic solvents [18].

Given that cellulose and CD are comprised of glucose units, and that native cellulose, like the native CD, has limited enantioselectivity when compared to derivatized cellulose under normal phase conditions, it was reasonable to consider that an analogous situation might exist with native CDs [19].

Various isocyanate derivatives of the CD have been prepared and their chromatographic characteristics in the normal phase mode explored [14,19]. Of all the derivatized CD phases prepared, the naphthylethyl carbamate (NEC) derivative seemed to be the most versatile and exhibited chromatographic characteristics similar to the naphthylvaline type column

developed by Pirkle [20]. With the derivatized CD, there exists new sites for π-π interactions not available with the native CD. The carbamate linkage also introduces new possibilities for hydrogen bonding and dipole stacking interactions as well as additional stereogenic centers. Results from chromatographic studies of (R-), (S-), and racemic naphthylethyl derivatized ß-CD in the normal-phase mode indicate that, in many cases, chiral recognition had contributions from both the CD and substituent [19]. Inclusion complexation with the CD cavity is not thought to be a factor for chiral recognition in the normal-phase mode, because of competition from the mobile phase for the cavity. It was also observed that crowding of the substituents on the CD could also effect enantioselectivity [14].

In the case of the native CD, the ß-CD has been most widely used. The native γ-CD has not been as widely used because it is thought to be too large for "tight" inclusion complexation. In addition, γ-CD is thought to be more lightly loaded onto the silica gel sorbent than ß-CD, presumably because of the bulkiness of the γ-CD. Likewise, with derivatized CDs, attention has been focused on derivatized ß-CDs. The purpose of this study was to examine the (S)-(+)-naphthylethyl derivatized γ-CD based stationary phase. Because of the γ-CD's significantly larger size, it is possible that the derivatized γ-CD may have unique enantioselectivity.

EXPERIMENTAL

Chemicals. The various amino acids and the 3,5 dinitrobenzoyl chloride derivatizing reagent were obtained from Aldrich

TABLE I
DATA FOR BONDED SORBENT

%C_{link} [1]	%$C_{\gamma\text{-CD}}$ [2]	%C_{NEC} [3]	Coverage[4] (μmol/m^2)	Degree of Substitution (#NEC / CD)
2.62	3.52	5.77	0.1	9

[1]%C due to linkage
[2]%C due to linkage and γ-CD
[3]%C due to linkage, γ-CD, and (S)-NEC
[4]amount of γ-CD on sorbent

(Milwaukee, WI). Absolute ethanol was obtained from Quantum (Tuscola, IL). All other solvents were all obtained from Fischer Scientific (St.Louis, MO). For each sample, about 10 mg was reacted with an excess amount of 3,5 dinitrobenzoyl chloride in acetone. The mixture was heated to 80° C for 20 minutes. The acetone was then evaporated off and the product was dissolved in ethanol (aliphatic amino acids) or acetonitrile (aromatic amino acids).

Preparation of the CD bonded stationary phase. The preparation of the CD bonded sorbent was accomplished according to Armstrong [21]. The (S)-(+)-naphthylethyl carbamate derivatization of the CD was also described previously [11]. The bonded sorbents were then slurry packed into a 250 X 4.6 mm column. A sample of the bonded sorbents was sent out for carbon analysis (Galbraith Laboratories, Inc., Knoxville, TN). The results are reported in Table I.

Equipment. The HPLC system used for this experiment consisted of a Shimadzu LC-600 and a SPD-6A UV detector interfaced to a Chromatopac CR-501 data station. The flow rate was 1 ml/min throughout the experiment and sample detection was accomplished at 254 nm.

RESULTS AND DISCUSSION

Bonding: As can be seen from Table I, γ-CD coverage was about 0.1 μmol/m². Thus, it appears that γ-CD bonds at a lower surface concentration than the reported values for ß-CD (ca. 0.2-0.3 μmol/m²) [22]. The γ-CD, being larger, is more excluded than the smaller ß-CD from the pores. In addition, initial attachment of γ-CD could locally sterically restrict further addition of γ-CD.

Substitution of the naphthylethyl substituents: Although the substitution pattern on the CD is not known, it is thought that substitution occurs predominantly on the secondary hydroxyls because the CD is attached to the silica substrate through the primary hydroxyls. Generally, the degree of substitution on the γ-CD was higher than that reported for the ß-CD [19]. This is attributed to the larger number of sites available for substitution on the γ-CD (e.g., 16 sites on the γ-CD, 14 sites on the ß-CD). It is also probable that the substituents are less crowded on the larger γ-CD than on the smaller ß-CD.

Chromatographic results: A total of 11 amino acids were derivatized with the 3,5-dinitrobenzoyl chloride. All

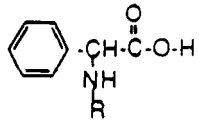
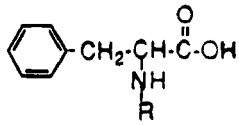
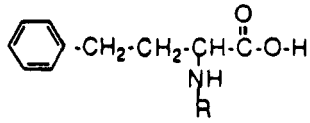
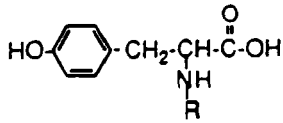
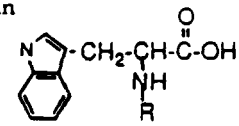
TABLE II
CHROMATOGRAPHIC DATA FOR THE SEPARATION OF THE
3,5 DINITROBENZOYL CHLORIDE DERIVATIVES OF ALIPHATIC AMINO ACIDS[1]

COMPOUND		k'	α	Rs
DL-alanine	$CH_3-\underset{\underset{R}{|}}{\overset{\overset{NH}{|}}{CH}}-COOH$	0.65^L	1.15	0.8
DL-norvaline	$CH_3-CH_2-\underset{\underset{R}{|}}{\overset{\overset{NH}{|}}{CH}}-COOH$	0.47^L	1.18	0.8
DL-norleucine	$CH_3-CH_2-CH_2-\underset{\underset{R}{|}}{\overset{\overset{NH}{|}}{CH}}-COOH$	0.42^L	1.24	1.0
DL-valine	$CH_3-\underset{\underset{R}{|}}{\overset{\overset{CH_3NH}{|}}{CH}}-CH-COOH$	0.44^L	1.16	0.7
DL-leucine	$CH_3-\underset{CH_3}{CH}-CH_2-\underset{\underset{R}{|}}{\overset{\overset{NH}{|}}{CH}}-COOH$	0.52^L	1.21	1.1
DL-isoleucine	$CH_3-CH_2-\underset{\underset{R}{|}}{\overset{\overset{CH_3\,NH}{|}}{CH}}-CH-COOH$	0.42^L	1.17	0.5

[1]mobile phase condition was 50:50 (v/v) ethanol:acetonitrile

chromatographic data (i.e. capacity factor (k'), selectivity (α), and resolution (R_s)) are presented in Tables II and III. The superscript on the capacity factor indicates the configuration of the enantiomer that eluted first. The aliphatic amino acids were chromatographed using 50:50 ethanol/acetonitrile. Separation of the aromatic amino acids were achieved using 99:1 ethanol/acetic acid as the mobile phase. A typical chromatogram showing the

TABLE III
CHROMATOGRAPHIC DATA FOR THE SEPARATION OF THE
3,5 DINITROBENZOYL CHLORIDE DERIVATIVES OF AROMATIC AMINO ACIDS[1]

COMPOUND	k'	α	Rs
DL-phenylglycine 	25.65[b]	1.40	7.1
DL-phenylalanine 	11.50[b]	1.20	3.2
DL-homophenylalanine 	8.47[b]	1.09	1.1
DL-tyrosine 	10.85[b]	1.27	3.9
DL-tryptophan 	12.05[b]	1.32	3.7

[1]mobile phase condition was 99:1 (v/v) ethanol:acetic acid

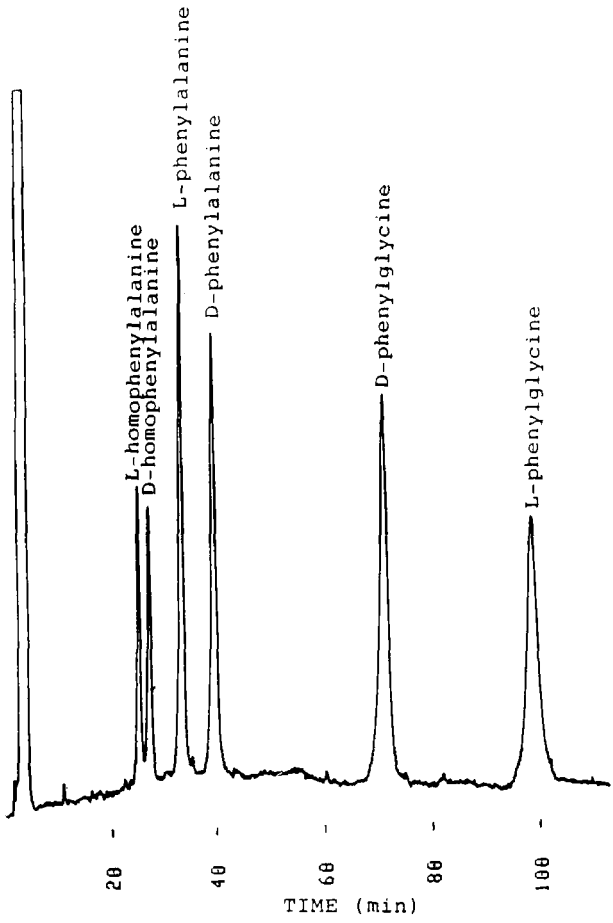


Figure 1. Separation of a mixture of 3,5-dinitrobenzoyl chloride derivatived DL-aromatic amino acids on a 25 cm x 4.6 mm I.D. (S)-naphthylethylcarbamoylated- γ-cyclodextrin column. Mobile phase conditions were 99:1 ethanol/acetic acid (v:v).

separation of racemic mixtures of aromatic amino acids is illustrated in Figure 1.

Retention: The retention characteristics seemed to display the same type of trend previously reported for the (S)-naphthylethyl carbamoylated ß-CD [19]. That is, within a class of compounds

(i.e. aromatic amino acids, or aliphatic amino acids), capacity factors increased as the carbon chain length decreased. For instance, phenylglycine had a k' of 25.65 while homophenylalanine had a k' of 8.47 and phenylalanine had a k' of 11.50. Likewise, alanine had a larger k' (0.65) than both norvaline (0.47) and norleucine (0.42). This may be attributed to the increased organic character, therefore, increased solubility of the solute in the mobile phase, that occurs with the lengthening of the carbon chain. Hence, larger compounds are thought to be distributed more into the nonpolar mobile phase rather than into the CSP.

Although the trends within groups were similar, the actual capacity factors achieved on the derivatized γ-CD column were sometimes significantly different from those previously reported for the naphthylethyl derivatized ß-CD column [19]. Under the same mobile phase conditions as was used for the work on the analogous derivatized ß-CD column (e.g. 99:1 MeOH/acetic acid), it was observed that the aromatic amino acids were retained longer on the γ-column [19]. This is somewhat surprising, considering the fact that the surface concentration of γ-CD is generally lower than that obtained for the ß-CD. The longer retention on the derivatized γ-CD may be due to more effective π-π interactions between the solute and the CD substituents on the derivatized γ-CD than on the derivatized ß-CD, because the substituents on the γ-CD are less congested than on the ß-CD.

Selectivity: In general, as the carbon chain length increased on the aliphatic amino acids, so did the selectivity (see Figure 2). Leucine and valine follow a similar trend, with leucine

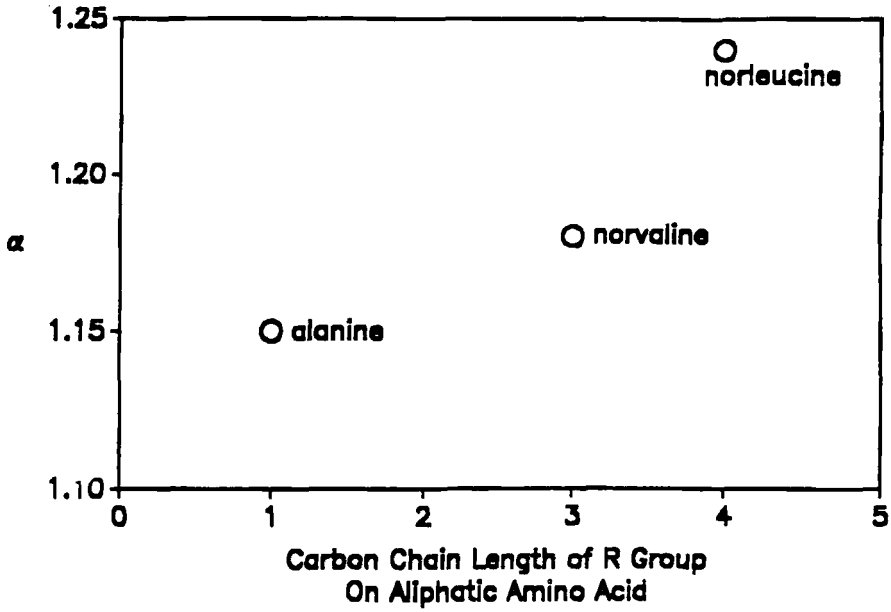


Figure 2. Plot illustrating effect of chain length on selectivity (α) for 3,5-dinitrobenzoyl chloride derivatized DL-aliphatic amino acids.

exhibiting a higher selectivity than valine. It is interesting to note, however, that the selectivities obtained for valine and isoleucine were similar. The selectivities obtained in this study for the aliphatic amino acids were comparable or possibly slightly better than those obtained on the analogous derivatized ß-CD column [19].

The aromatic amino acids, on the other hand, exhibited decreased selectivity with increasing chain length as depicted in Figure 3. Phenylglycine had the best selectivity (1.40). The lowest selectivity (1.09) was observed for homophenylalanine. Studies done in the reversed phase mode on native cyclodextrin stationary phases reported that the highest selectivities were

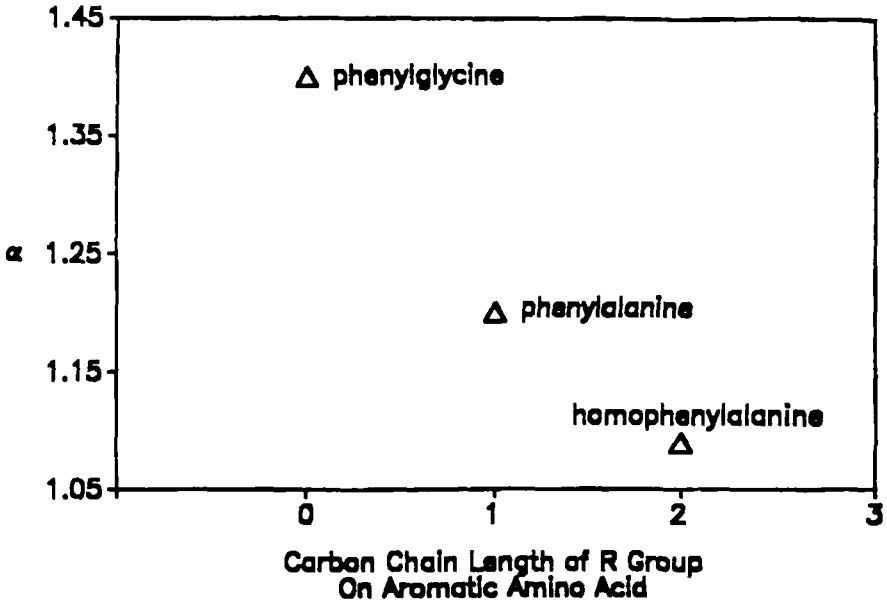


Figure 3. Plot illustrating effect of chain length on selectivity (α) for 3,5-dinitrobenzoyl chloride derivatized DL-aromatic amino acids.

obtained when the stereogenic center was positioned between two aromatic rings and that chiral recognition lessened as the aromatic rings became more distant from the stereogenic center [23]. This trend seems to hold for the derivatized aromatic amino acids tested on this column as well. Three out of the five aromatic amino acids (phenylglycine, homophenylalanine, and tyrosine) tested on the (S)-naphthylethyl derivatized γ-CD column had selectivities that were slightly lower than that reported previously for the (S)-naphthylethyl derivatized ß-CD column [19]. On both columns, the highest selectivity obtained for the aromatic amino acids was observed for phenylglycine.

Elution order: For all of the aliphatic amino acids, the L enantiomer came out first. The same enantiomeric elution order was reported for the (S)-NEC ß-CD column [19]. This is a good indicator that similar chiral recognition mechanisms are involved on both columns.

However, the aromatic amino acids provide anomalous results. On the derivatized γ-CD column, the L isomer eluted first for all of the aromatic compounds except for phenylglycine. In contrast, on the analogous derivatized ß-CD column, the L enantiomer was observed to elute first for all of the aromatic amino acids except for homophenylalanine [19]. This suggests that although similar chiral recognition mechanisms are operative on the NEC ß-CD and NEC γ-CD columns, there are important differences giving rise to the unique chromatographic characteristics of the two stationary phases.

Conclusions: It is clear that more extensive investigations of the chiral recognition mechanism on these derivatized CD phases are warranted. From the chromatographic data, it seems likely that the enantioselective mechanism works similarly for both the derivatized γ-CD column as well as the derivatized ß-CD column however, it is apparent that differences do exist. Currently, intensive spectroscopic and additional chromatographic experiments are being conducted to help reveal the intermolecular mechanics that are important for the chiral recognition mechanisms of these new derivatized cyclodextrin phases.

ACKNOWLEDGEMENTS

The authors wish to thank the American Chemical Society Petroleum Research Fund, the National Science Foundation and the

Pacific Health Research Institute for their generosity and support of this project.

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Received: May 29, 1992
Accepted: August 11, 1992