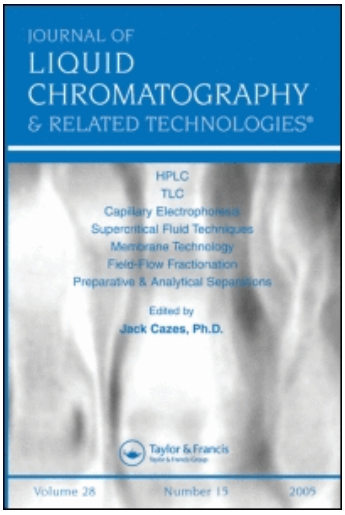


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# Liquid Chromatographic Enantiomer Separation of *N*-Phthaloyl Protected α-Amino Acids on Coated and Immobilized Chiral Stationary Phases Derived from Polysaccharide Derivatives

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**Abstract:** Liquid chromatographic enantiomer separation of *N*-phthaloyl (PHT) protected α-amino acids on several coated and immobilized chiral stationary phases (CSPs) derived from polysaccharide derivatives was performed. The coated CSP of Chiralpak AD showed more or less enantioseparation than the covalently bonded CSP of Chiralpak IA with the same chiral selector of amylose tris(3,5-dimethylphenylcarbamate). However, the coated Chiralcel OD showed greater enantioseparation than the covalently bonded Chiralpak IB with the same chiral selector of cellulose tris(3,5-dimethylphenylcarbamate). Among all examined CSPs, Chiralcel OD afforded the greatest performance for enantiomer resolution of *N*-PHT α-amino acids and, therefore, all analytes enantiomers were baseline separated on Chiralcel OD. The chromatographic method developed in this study was usefully applied for

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determination of the enantiomeric purity of commercially available *N*-PHT α-amino acids analytes.

**Keywords:** Enantiomer separation, *N*-Phthaloyl α-amino acids, Chiral stationary phase



## INTRODUCTION

*N*-Protected α-amino acids in the fields of pharmaceutical chemistry and biochemistry have been extensively used as important chiral building blocks of peptides and proteins.[1,2] Consequently, the studies of the determination of enantiomeric purity of *N*-protected α-amino acids have been of great interest, and many techniques for these determinations have been developed and employed.[3] Among several *N*-protecting groups for α-amino acids, the *N*-phthaloyl (PHT) group is attractive in certain instances,[4] because it has often been used for protecting them moiety of not only α-amino acids but also primary amine compounds, and it can be readily removed under mild reaction conditions of hydrazine reagents.[5,6] Also the *N*-PHT α-amino acid derivatives have been usefully employed as chiral auxiliaries or chiral resolving agents for asymmetric synthesis.[7–9] Also, it has been reported that the *N*-PHT group as a chromophoric derivative of the α-amino acids derivatives can be applied for circular dichroism studies.[10] In spite of these chiral potential utilities of the *N*-PHT α-amino acid acids, very few results for enantiomer separation of *N*-PHT α-amino acids have been reported.[11,12] For example, the enantioseparation of five *N*-PHT α-amino acids, as well as their ester and amide derivatives, has been performed on Pirkle-type CSP with reasonable separation factors.[11] On macrocyclic antibiotic, ristocetin A bonded CSP, enantiomer resolution of only two analytes of *N*-PHT methionine and *N*-PHT α-amino-n-butyric acid has been reported.[12] In this study, we present the liquid chromatographic enantiomer resolution of several *N*-PHT protected α-amino acids on several coated and immobilized CSPs derived from polysaccharide derivatives.[13–15]

## EXPERIMENTAL

Chromatography was performed at room temperature using an HPLC consisting of a Waters model 510 pump, a Rheodyne model 7125 injector with a 20 μL loop, a variable wavelength UV detector (Waters 484), and an HP 3396 series II recorder. All CSPs columns were purchased from Daicel Chemical Company (Tokyo, Japan). HPLC grade hexane and 2-propanol were obtained from J. T. Baker (Phillipsburg, NJ). Trifluoroacetic acid (TFA) was obtained from Aldrich (Milwaukee, WI). The *N*-PHT racemic and L-α-amino acids were prepared according to the reported procedures.[2,16] *N*-PHT L-glutamic acid and L-phenylalanine were obtained from Fluka company.

## RESULTS AND DISCUSSION

Tables 1 and 2 show chromatographic results for the separation of the enantiomers of *N*-PHT $\alpha$-amino acids, not only on the most commonly used coated CSPs such as Chiralcel OD, Chiralpak AD, Chiralpak AS, and Chiralcel OF[17,18] but also on the covalently CSPs, Chiralpak IA, and Chiralpak IB.[13–15] Among all examined CSPs, in general, Chiralcel OD, the cellulose tris(3,5-dimethylphenylcarbamate) coated type CSP showed the greatest separation factor ($\alpha = 1.10–2.64$) and, therefore, all investigated *N*-PHT $\alpha$-amino acids enantiomers were baseline separated on Chiralcel OD, whereas Chiralpak AS showed the smallest separation factor. In Table 2, Chiralcel OF shows generally lower performance than Chiralcel OD, however, it provided baseline separation except for two analytes. Also, Chiralcel OD, the cellulose tris(3,5-dimethylphenylcarbamate) coated CSP showed generally greater enantioselectivities than Chiralpak AD, the amylose tris(3,5-dimethylphenylcarbamate) coated CSP in Table 1. Similarly, Chiralpak IB, the immobilized CSP of the same chiral selector of Chiralcel OD showed generally better performance than Chiralpak IA, the immobilized CSP of the same chiral selector of Chiralcel AD, as shown in Table 3. Although, the separation factors of all analytes on the covalently bonded Chiralpak IB showed lower enantioseparation than those on the coated Chiralcel OD with the same chiral selector, all analytes except for two cases (entries 5 and 10) provided quite good enantioselectivities ($\alpha = 1.13–1.73$) on Chiralpak IB, using 2-propanol in hexane with 0.1% TFA as a mobile phase.[14,15] However, Chiralpak IA of the covalently bonded CSP showed more or less enantioseparation than Chiralpak AD of the coated CSP with the same chiral selector.[13,15]

It is noted that the L-enantiomers of all examined *N*-PHT $\alpha$-amino acids are selectively retained on Chiralcel OD, Chiralpak AS, Chiralcel OF, and Chiralpak IB, however, the elution orders of enantiomer separation of *N*-PHT $\alpha$-amino acids are not always consistent on Chiralpak AD and Chiralpak IA derived from amylose tris(3,5-dimethylphenylcarbamate). It is considered that more than one chiral recognition process between *N*-PHT $\alpha$-amino acid analyte and the chiral selector of amylose tris(3,5-dimethylphenylcarbamate) might be involved. Contrary to Chiralcel OD and Chiralpak IB, the D-enantiomers of all examined analytes except for *N*-PHT glutamic acid are selectively retained on Chiralpak IA and, therefore, these CSPs in Tables 1 and 3 might be complementarily used for the reversal of elution order of the determination of enantiomeric purity.[19] The chromatographic method developed in this study was used for determination of the enantiomeric purity of two commercially available reagents of *N*-PHT L-glutamic acid and *N*-PHT L-phenylalanine. The enantiomeric impurities of 0.4% for these two samples were determined on Chiralcel OD. Chromatograms of determination of the enantiomeric purity of these analytes are presented in Figures 1 and 2.

In conclusion, we demonstrated the liquid chromatographic separation of enantiomers of *N*-PHT protected $\alpha$-amino acids on several coated and

***Table 1.*** Enantiomer separation of *N*-PHT $\alpha$-amino acids on Chiralpak AD and Chiralcel OD

| | | Chiralpak AD | | | | Chiralcel OD | | | |
|---|---|---|---|---|---|---|---|---|---|
| Entry | Analyte | $\alpha^a$ | $k_1'^b$ | Rs[c] | Conf.[d] | $\alpha^a$ | $k_1'^b$ | Rs[c] | Conf.[d] |
| 1 | 2-Aminobutyric acid | 1.03 | 8.87 | 0.27 | D | 2.01 | 3.96 | 7.60 | L |
| 2 | 2-Aminocaprylic acid | 1.00 | 6.93 | – | | 1.91 | 2.70 | 9.70 | |
| 3 | Alanine | 1.05 | 12.09 | 0.71 | L | 1.47 | 4.27 | 4.55 | L |
| 4 | Glutamic acid | 1.13 | 6.61[e] | 1.66 | L | 2.64 | 6.78[e] | 8.49 | L |
| 5 | Isoleucine | 1.08 | 7.94 | 1.32 | L | 1.10 | 2.44 | 0.95 | L |
| 6 | Leucine | 1.31 | 5.93 | 4.34 | D | 1.20 | 3.87 | 1.96 | L |
| 7 | Methionine | 1.07 | 14.65 | 1.22 | D | 1.90 | 6.68 | 7.02 | L |
| 8 | Norleucine | 1.13 | 6.58 | 1.89 | D | 1.76 | 3.05 | 5.82 | L |
| 9 | Norvaline | 1.00 | 8.36 | – | | 2.11 | 3.13 | 7.40 | L |
| 10 | Phenylalanine | 1.86 | 10.44 | 9.72 | D | 1.38 | 8.25 | 3.08 | L |
| 11 | Phenylglycine | 1.28 | 16.32 | 4.03 | D | 1.76 | 7.52 | 5.52 | L |
| 12 | Valine | 1.16 | 8.77 | 2.32 | L | 1.34 | 2.91 | 2.84 | L |

Mobile phase; 5% 2-propanol/hexane (V/V) containing 0.1% TFA; Detection UV 254 nm, Flow rate = 1 mL/min.

[a]Separation factor.

[b]Capacity factor for the first eluted enantiomer.

[c]Resolution factor.

[d]Indicates the absolute configuration of the second eluted enantiomer.

[e]10% 2-propanol/hexane (V/V) containing 0.1% TFA.

***Table 2.*** Enantiomer separation of *N*-PHT $\alpha$-amino acids on Chiralpak AS and Chiralcel OF

| | | Chiralpak AS | | | | Chiralcel OF | | | |
|---|---|---|---|---|---|---|---|---|---|
| Entry | Analyte | $\alpha$[a] | $k_1'$[b] | Rs[c] | Conf.[d] | $\alpha$[a] | $k_1'$[b] | Rs[c] | Conf.[d] |
| 1 | 2-Aminobutyric acid | 1.16 | 4.44 | 1.29 | L | 1.00 | 9.22 | – | |
| 2 | 2-Aminocaprylic acid | 1.00 | 3.71 | – | | 1.74 | 3.67 | 2.67 | |
| 3 | Alanine | 1.36 | 6.43 | 3.26 | L | 1.44 | 8.05 | 2.54 | L |
| 4 | Glutamic acid | 1.41 | 11.35[e] | 2.17 | L | 1.37 | 9.68[e] | 1.53 | L |
| 5 | Isoleucine | 1.41 | 3.09 | 3.24 | L | 2.17 | 3.33 | 4.15 | L |
| 6 | Leucine | 1.00 | 3.30 | – | | 1.93 | 3.40 | 3.24 | L |
| 7 | Methionine | 1.00 | 11.21 | – | | 1.56 | 5.16[e] | 2.35 | L |
| 8 | Norleucine | 1.07 | 4.14 | 0.40 | L | 1.77 | 4.57 | 3.18 | L |
| 9 | Norvaline | 1.15 | 4.34 | 1.25 | L | 1.82 | 4.92 | 3.42 | L |
| 10 | Phenylalanine | 1.00 | 8.57 | – | | 1.60 | 4.16[e] | 2.17 | L |
| 11 | Phenylglycine | 1.35 | 9.25 | 2.35 | L | 1.07 | 7.96[e] | 0.36 | L |
| 12 | Valine | 1.66 | 3.97 | 3.72 | L | 2.05 | 3.87 | 3.88 | L |

Mobile phase; 5% 2-propanol/hexane (V/V) containing 0.1% TFA; Detection UV 254 nm, Flow rate = 1 mL/min.

[a]Separation factor.

[b]Capacity factor for the first eluted enantiomer.

[c]Resolution factor.

[d]Indicates the absolute configuration of the second eluted enantiomer.

[e]10% 2-propanol/hexane (V/V) containing 0.1% TFA.

***Table 3.*** Enantiomer separation of *N*-PHT $\alpha$-amino acids on Chiralpak IA and Chiralpak IB

| | | Chiralpak IA | | | | Chiralcel IB | | | |
|---|---|---|---|---|---|---|---|---|---|
| Entry | Analyte | $\alpha^a$ | $k_1'^b$ | Rs[c] | Conf.[d] | $\alpha^a$ | $k_1'^b$ | Rs[c] | Conf.[d] |
| 1 | 2-Aminobutyric acid | 1.08 | 6.47 | 1.23 | D | 1.39 | 2.91 | 4.29 | L |
| 2 | 2-Aminocaprylic acid | 1.09 | 5.24 | 1.31 | | 1.16 | 1.96 | 2.00 | |
| 3 | Alanine | 1.00 | 8.06 | – | | 1.15 | 3.44 | 1.84 | L |
| 4 | Glutamic acid | 1.07 | 5.86[e] | 0.74 | L | 1.73 | 5.14[e] | 5.63 | L |
| 5 | Isoleucine | 1.10 | 6.03 | 1.46 | D | 1.07 | 1.99 | 0.81 | L |
| 6 | Leucine | 1.33 | 4.56 | 4.95 | D | 1.12 | 2.25 | 1.52 | L |
| 7 | Methionine | 1.14 | 10.35 | 2.20 | D | 1.32 | 5.02 | 3.75 | L |
| 8 | Norleucine | 1.19 | 4.94 | 2.79 | D | 1.27 | 2.28 | 2.57 | L |
| 9 | Norvaline | 1.12 | 5.83 | 1.85 | D | 1.41 | 2.52 | 5.11 | L |
| 10 | Phenylalanine | 1.63 | 7.66 | 8.70 | D | 1.00 | 5.32 | – | |
| 11 | Phenylglycine | 1.35 | 11.65 | 4.73 | D | 1.25 | 5.39 | 2.55 | L |
| 12 | Valine | 1.04 | 6.48 | 0.54 | D | 1.13 | 2.18 | 1.57 | L |

Mobile phase; 5% 2-propanol/hexane (V/V) containing 0.1% TFA; Detection UV 254 nm, Flow rate = 1 mL/min.

[a]Separation factor.

[b]Capacity factor for the first eluted enantiomer.

[c]Resolution factor.

[d]Indicates the absolute configuration of the second eluted enantiomer.

[e]10% 2-propanol/hexane (V/V) containing 0.1% TFA.

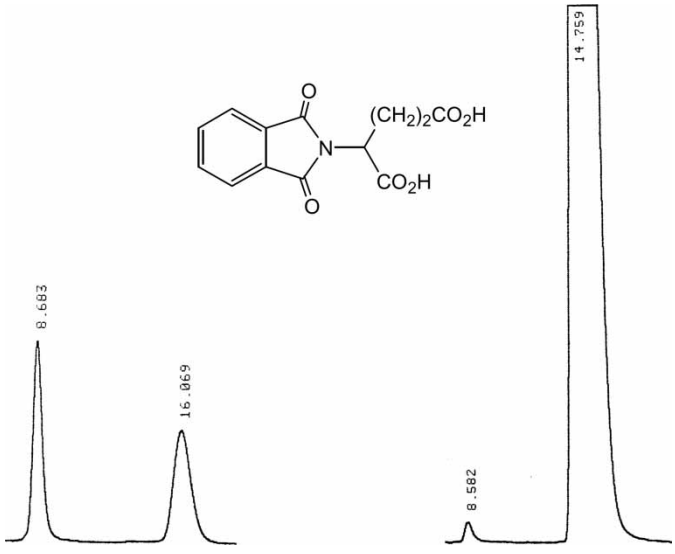


***Figure 1.*** Chromatograms of enantiomer separation of racemic *N*-PHT glutamic acid (the left) and *N*-PHT L-glutamic acid (Fluka reagent) (the right, D:L = 0.4: 99.6) on Chiralcel OD; Mobile phase: 20% 2-propanol/hexane (V/V) containing 0.1% TFA; Flow rate = 1 mL/min; UV 254 nm; Injected amount 20–100 μg.

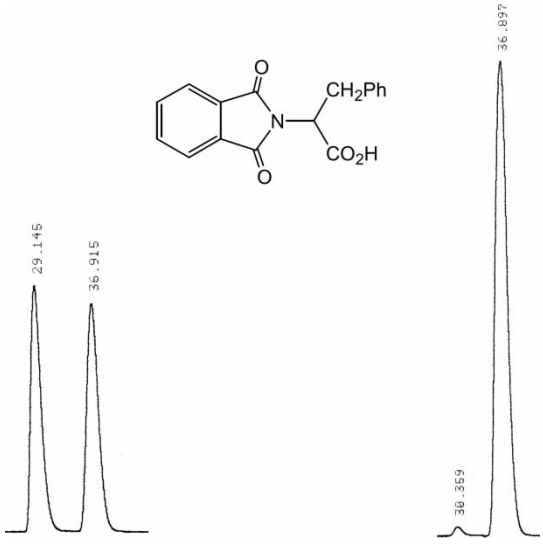


***Figure 2.*** Chromatograms of enantiomer separation of racemic *N*-PHT phenylalanine (the left) and *N*-PHT L-phenylalanine (Fluka reagent) (the right, D:L = 0.4: 99.6) on Chiralcel OD; Mobile phase: 5% 2-propanol/hexane (V/V) containing 0.1% TFA; Flow rate = 1 mL/min; UV 254 nm; Injected amount 40–60 μg.

immobilized CSPs derived from polysaccharide derivatives. This is the first reported for the enantiomer resolution of several *N*-PHT $\alpha$-amino acids using polysaccharide derived CSPs. Among all examined CSPs in this study, the cellulose tris(3,5-dimethylphenylcarbamate) derived CSPs, Chiralcel OD and Chiralpak IB showed quite good enantioselectivities. Especially, since Chiralcel OD showed excellent resolving ability for the enantiomer resolution of all *N*-PHT $\alpha$-amino acids, it is expected to be quite useful for determination of the enantiomeric purity of these analytes and their related compounds.

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