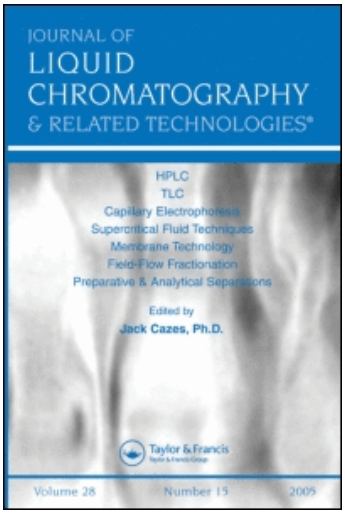

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RETENTION AND SELECTIVITY OF AMINO ACID ESTER DERIVATIVES ON (R)-N-(3,5-DINITROBENZOYL)-PHENYLGLYCINE COLUMN

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ABSTRACT

The effect of the size of alkyl substituent (ethyl, 2-propyl, 1-butyl or 2-butyl) in the ester group of benzoyl derivatives of amino acids on the separation factor and retention of enantiomers was examined. Pirkle type chiral stationary phase (R)-3,5-dinitrobenzoylphenylglycine) and binary nonaqueous eluents were used. The Snyder-Soczewiński linear equation was applied for presentation of retention vs. eluent composition relationships, which permits the evaluation of separation selectivity in a wide range of concentrations of the more polar component in the binary mobile phase.

It was found that, in the majority of cases, the best separation was obtained for 2-propyl derivatives of amino acids, although some exceptions from this rule were also observed. Similarly, in some cases, the ethyl derivatives of amino acids do not exhibit the strongest retention.

Both selectivity, as well as retention, depended on the type and concentration of the more polar component of the eluent.

INTRODUCTION

Among the hundreds of chiral stationary phases (CSP), the chiral recognition mechanism on a Pirkle type of π donor- acceptor phases has been studied intensively, and reviewed.[1-4] It was proposed that the separation of enantiomers on these CSPs is dependent on the formation of diastereomeric complexes between the CSP and enantiomers to be separated. The formation of these diastereomeric complexes requires enantioselective interactions, which are formed by an appropriate combination of suitable functional groups (ligands) on CSP with complementary functional groups on the molecules of enantiomers.

Pirkle and Pochapsky[2] suggested that for resolution to occur there must be at least three simultaneous interactions between the CSP and one of the analyte enantiomers, one or more of these interactions being stereochemically dependent. The π donor- acceptor phases use combinations of hydrogen bonds, dipole – dipole, π - π and steric interactions to achieve chiral recognition.

Pirkle and Pochapsky[2] also suggested that enantioselective adsorption of enantiomers implies that the CSP "senses" the spatial relationships between structural elements of the analytes. This is why derivatization in chromatographic chiral separation of chiral samples can improve selectivity and, is in many cases, necessary.

Diastereomeric amides, carbamates, and ureas[1] usually show better selectivity than non-derivatized samples, which, can be connected with the possibility of the hydrogen bonding and dipole – dipole interactions between the chiral stationary phase and enantiomers. In this case, simultaneous intermolecular forces between two enantiomers and CSP functional groups play a decisive role in enantioselectivity.

Depending on the derivatizable functional group the chiral derivatizing reagent is selected with the goal of performing a reaction with 100% yield, with no noticeable racemization of the chiral centres of the investigated compounds.

The present study was focused on the influence of the steric interaction provided by the size of the esters groups in derivatized amino acids moieties. A series of seven amino acids esters containing ethyl, 2-propyl, 1-butyl, or 2-butyl groups were prepared and investigated.

EXPERIMENTAL

Apparatus

The chromatographic system consisted of a Knauer HPLC pump 64 and a Rheodyne (Cotati, CA., USA) injector valve for manual injection. A photo-

metric UV detector was set at 254 nm. The column used was (R)- N – 3,5-(dinitrobenzoyl) phenylglycine (25 x 0.4 cm, $d_{p.}$ = 5 μm.) from Supelco Inc. (Bellefonte, PA, USA). The binary mobile phases were prepared from solvents of HPLC grade.

Materials and Reagents

Enantiomers and racemates of amino acids were obtained from Sigma (St. Louis, MO, USA).

Mobile phase components: 1-propanol, 2-propanol, and n-hexane were HPLC- grade and obtained from Fluka, Sigma – Aldrich Chemie GmbH (Steinheim, Germany); ethanol was purchased from Polmos, Poland.

For derivatization procedure: 2-propanol, 1-butanol, 2-butanol, were obtained from Fluka, Sigma – Aldrich Chemie GmbH (Steinheim, Germany), benzoyl chloride from Merck (Darmstadt, Germany).

Derivatization Procedure

The derivatization procedure was carried out according to the method described previously by Pirkle and Perrin.[5] Solutions of amino acids were prepared at ca. 1 mg/ mL in the 1.25 M HCl in appropriate alcohol (ethanol, 2-propanol, 1-butanol, or 2-butanol). The samples of amino acids were dissolved separately in the proper alcohol to obtain ethyl-, 2-propyl, butyl-, or 2-butyl-esters. Samples were left at room temperature for a few hours. After this time, they were evaporated to dryness at 60°C.

Dry samples of amino acid esters were dissolved in dry tetrahydrofuran (THF) and benzoyl chloride and propylene oxide was added. Some problems arose when amino acids esters were dissolved in THF. In this case, after reaction with benzoyl chloride, it was necessary to remove traces of residual amino acid. Reaction mixtures were left for 15 minutes.

Next, they were filtered (special papers, FILTRAK GmbH, Germany) to remove traces of residual esters of amino acids. The solvent was evaporated under a stream of nitrogen. Residues were dissolved in the mobile phase.

RESULTS AND DISCUSSION

In order to evaluate the effect of the size of alkyl substituent (ethyl, 2-propyl, 1-butyl, and 2-butyl) in the ester group, benzoyl derivatives of some amino acids were used. The structure of CSP used can be found in the review.[2]

It was previously shown[6] that the Snyder- Soczewiński equation can be applied to describe the chromatographic behavior of organic compounds in a system: π-donor-acceptor chiral stationary phase (CSP) and nonaqueous organic mobile phase. With Snyder-Soczewiński's[7,8] approach, the following equation was applied to retention data obtained for DNBPG column:

$$\log k = \text{const} - m \log X_s \qquad (1)$$

where X_s is the mole fraction of the strong solvent in the binary mobile phase; the constant is equal to k value for the pure modifier S (for $X_s = 1.0$) and m is the slope of $\log k = f(\log X_s)$ plot.

The Snyder-Soczewiński equation, originally verified for polar sorbents, such as silica, alumina, and Florisil was also found to be valid for bonded stationary phases containing polar groups. Hurtubise,[9] Snyder, and Schunk[10] for aminopropyl phases, and Hara[11] for aminopropyl and cyanopropyl phases, obtained linear relationship between log k and log X_s.

From the practical point of view, the Snyder- Soczewiński equation is very useful because it allows investigation of the chromatographic behavior of analyte in wide ranges of the modifier concentration in the mobile phase, so, comparison of the analyte retention and changes of selectivity is easier and more exact.

One more example of such linear relationship of logarithm of the retention factor vs. logarithm of the mole fraction of polar modifier in the mobile phase, is given in Figure 1 where ethyl esters of some derivatized amino acids are chromatographed in the mobile phase composed of n-hexane and 2-propanol of different concentrations.

It is seen that at least two conclusions can be drawn from Figure 1:

Log k vs. log X_s relationships for a given pairs of enantiomers of the amino acids are sometimes not parallel, so in the case of separation of an amino acids mixture, different elution sequences can be expected.

The log k vs. log X_s for two enantiomers of a given racemate are, in several cases, not parallel and converge at higher concentrations of the mobile phase.

The differentiated slopes of the log k vs. log X_s indicate that the selectivity, and even sequence, of the peaks on chromatograms can change with mobile phase composition which is advantageous as far as optimisation of chromatographic analysis is concerned.

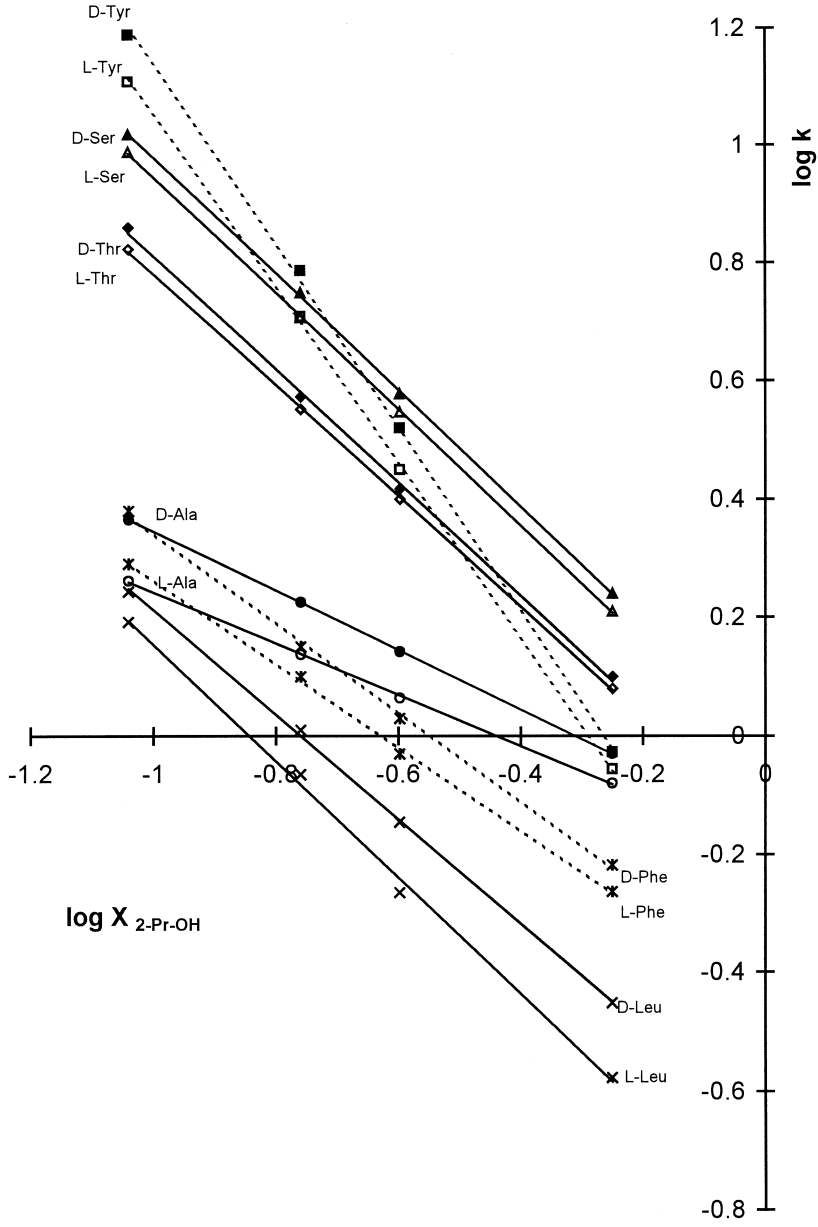

Figure 1. Relationships between log k values of ethyl esters of benzoyl derivatives of amino acids and logarithm of mole fraction of 2-propanol. Diluent: n-hexane. Stationary phase: (R)- 3,5- DNBPG. Flow rate: 1 mL/ min, Detection at 254 nm.

Taking into consideration the chemical structure of amino acids esters chromatographed, the butyl esters should be less strongly retained than, for example, ethyl esters. The reason for expected stronger adsorption (retention) of ethyl esters of amino acids results from weaker repulsive steric interactions of a small ethyl group in comparison to the bulky butyl group.

This conclusion, drawn from the considerations based on the three-point interactions model,[12] is only partly true because adsorption (retention) of different esters of the amino acid also depends on concentration of the modifier in the mobile phase.

It can be seen in Figures 1 and 2 that the lines of the log k vs. log X_s relationships for different esters (ethyl, 2-propyl, 1-butyl, and 2-butyl) of leucine enantiomers chromatographed in the same mobile phase cross each other. Similar effects were obtained both for 1-propanol (Figure 2) and 2-propanol (Figure 1) used as modifiers.

However, this effect (crossing lines of the log k vs. log X_s plots) is less pronounced in the case of tyrosine esters. The additional results obtained for, e.g., phenylalanine, alanine, serine, or threonine derivatives (not presented in this paper) confirm the conclusion that, not only the size of ester group influences the retention, but also the concentration and nature of modifier.

It is evident that molecules of polar modifier adsorbed on the CSP ligands play an important role in the adsorption process of amino acids enantiomers, resulting in stronger or weaker retention. It is surprising that, in a moderate range of elution strength (0.05-0.4 mole fraction) of the mobile phase, 2-propyl esters of derivatized leucine show a stronger adsorption (Figures 2 and 3) than ethyl esters. Retention of tyrosine esters is consistent with theoretical prediction and esters are adsorbed in the order: 2-butyl$\leq$1-butyl< 2-propyl< ethyl.

In fact, theoretical prediction that retention should increase with decreasing size of the ester group (decreasing steric hindrance) in amino acid molecules is true only at a very low concentration of modifier (X_s < 0.05 mole fraction).

Selectivity factors measured at low modifier concentrations, where usually the highest selectivity can be obtained, show that, in general, for bulky ester groups, e.g., 2-propyl or butyl, higher values of separation factors were obtained. There is one exception from this rule; the separation factor for ethyl esters of alanine enantiomers has the highest value for the mobile phase composed of n-hexane – 5% of 2-propanol (Figure 3).

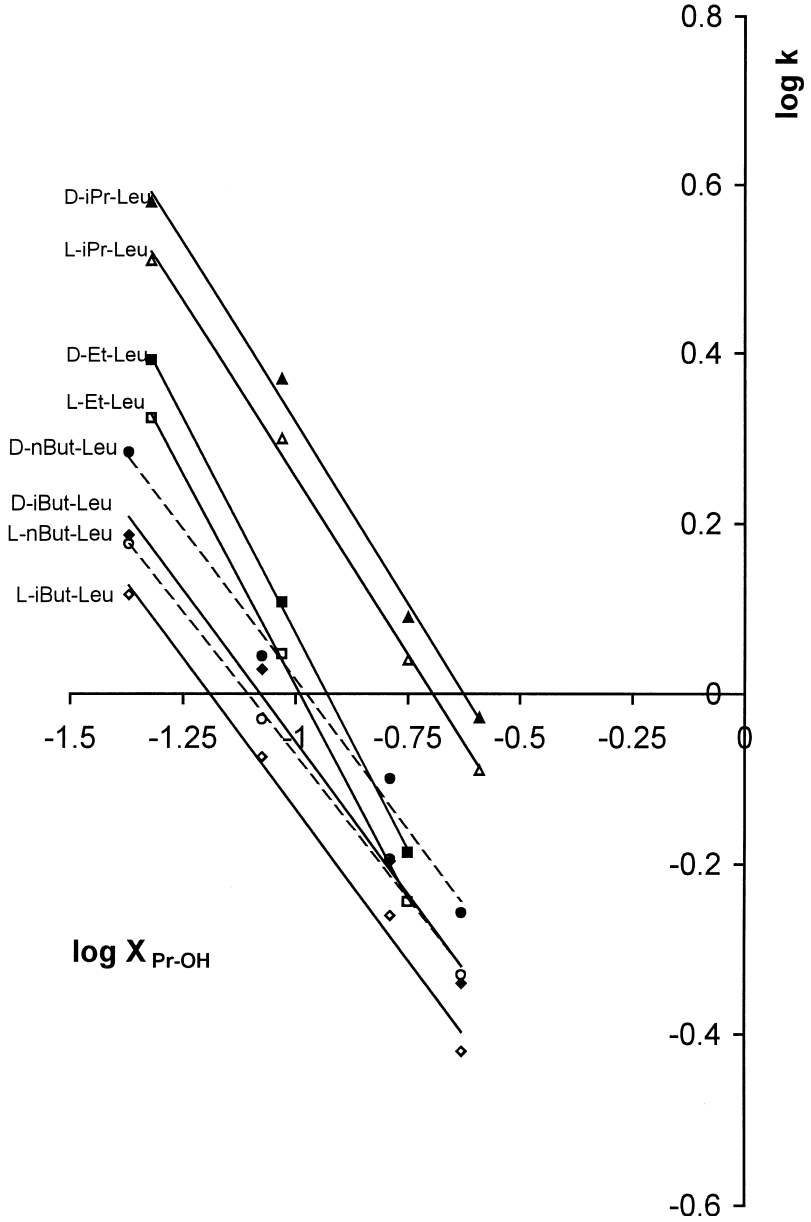

Figure 2. Relationships between log k values of different esters of benzoyl derivatives of leucine and logarithm of mole fraction of n-propanol. iPr – 2-propyl ester; Et- ethyl ester; nBut- 1-butyl ester; iBut- 2-butyl ester. Other conditions as in Figure 1.

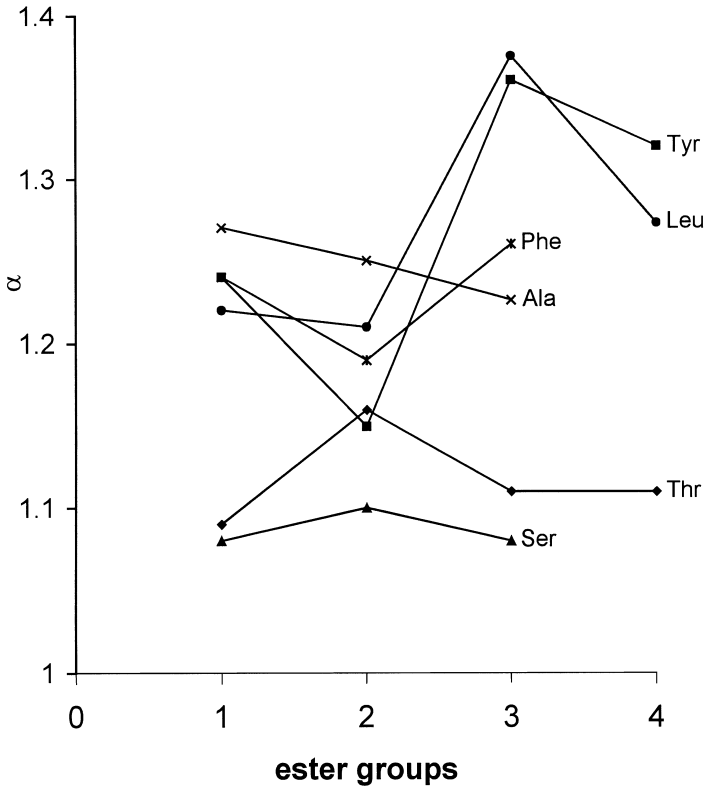

Figure 3. Influence of size of ester group on selectivity factor of amino acid enantiomer derivatives: 1) ethyl ester, 2) 2-propyl ester, 3) 1-butyl ester, and 4) 2-butyl ester. Conditions: mobile phase: 5% (v/v) 2-propanol + 95% (v/v) n-hexane. Flow 1 mL/ min, detection at 254 nm. Stationary phase: (R) – 3,5 – DNBPG.

This statement is not true when n-hexane- 5% 1-propanol is used as the mobile phase; in this case the separation factors for alanine enantiomers gradually increase in the order: ethyl, butyl, 2-propyl esters (Figure 4).

It is worthwhile to stress that both retention and selectivity strongly depend on the size of the ester groups in the amino acid molecule and type and concentration of the modifier in the mobile phase (compare Figures 3 and 4).

It is seen from Figures. 3 and 4, that there is no general rule how to optimise the chromatographic system for separation of enantiomers of derivatized

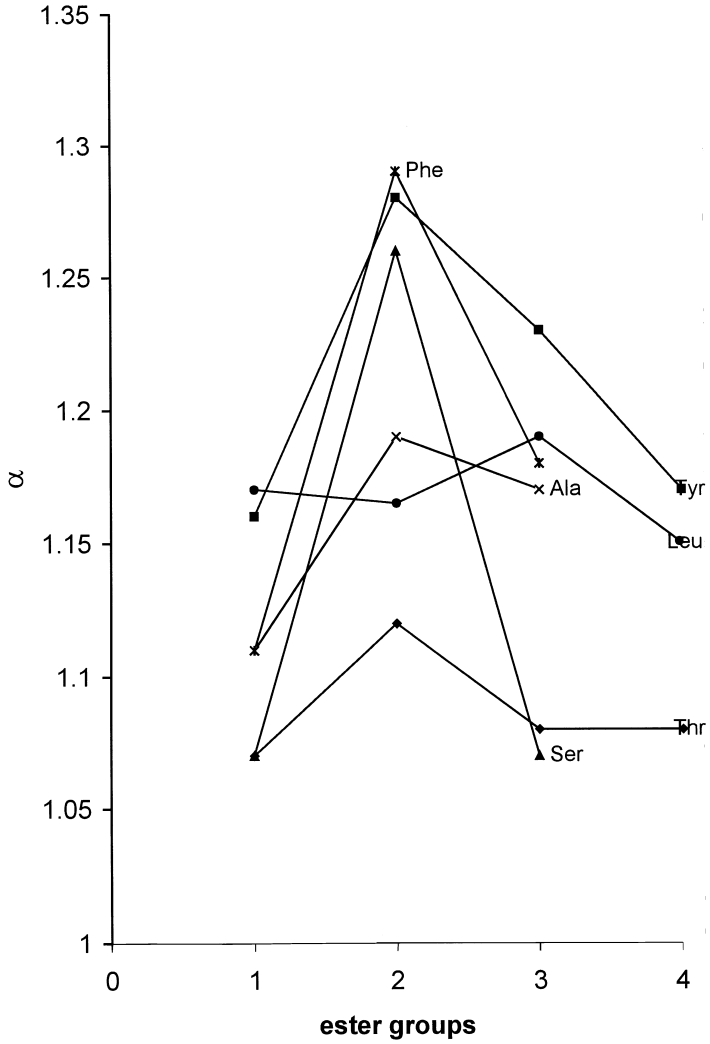

Figure 4. Influence of size of ester group on selectivity factor of amino acid enantiomer derivatives. 1) ethyl ester, 2) 2- propyl ester, 3) 1-butyl ester, 4) 2-butyl ester. Conditions: mobile phase: 5% (v/v) 1-propanol + 95% (v/v) n-hexane, flow 1 mL/ min, 254 nm detection; stationary phase (R) – 3,5 – DNBPG.

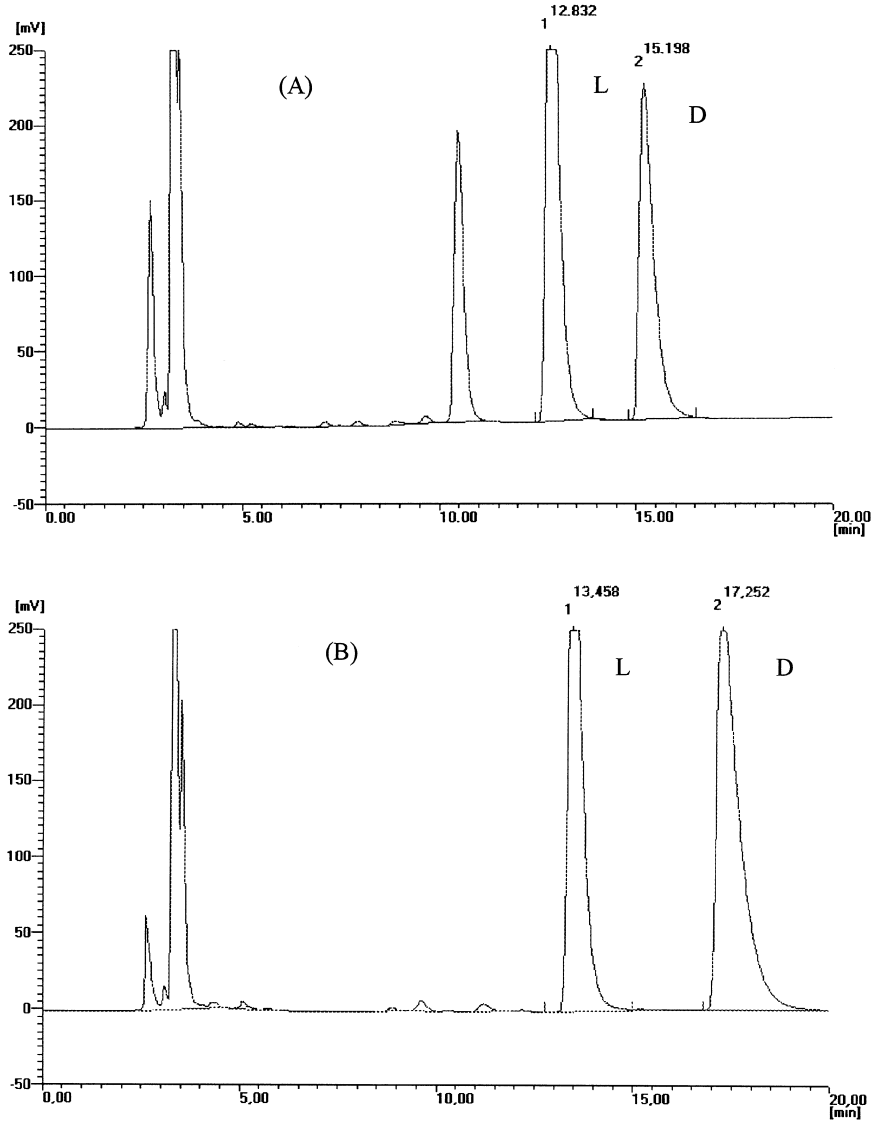

Figure 5. Chromatograms of the benzoyl derivatives of phenylalanine esters. (A)- ethyl esters and (B)- 2-propyl esters. Chromatographic conditions: stationary phase-(R)-N-(3,5-dinitrobenzoyl) phenylglycine; mobile phase: 5% of 2-propanol in hexane; Flow 1mL/min, UV detection at 254 nm.

amino acids because of the significant influence of the modifier type on the selectivity. The highest values of separation factors in 2-propanol –n-hexane (Figure 3) mobile phase were observed for butyl esters of tyrosine, leucine, and phenylalanine, 2-propyl esters of serine and threonine and ethyl ester of alanine.

When 2-propanol in the mobile phase was replaced by 1- propanol (Figure 4), the highest values of separation factors were obtained for 2-propyl esters (with the exception of leucine). It also seems that there is no practical reason to use alcohols with longer than butyl chain for esterification of amino acids.

A good illustration of the main conclusions drawn from the investigations carried out is separation of the ethyl (Figure 5A) and 2-propyl (Figure 5B) esters of the benzoyl derivatives of phenylalanine. Better separation was achieved for 2-propyl esters (separation factor $\alpha = 1.40$) in comparison to ethyl esters ($\alpha = 1.31$).

The modifier of the mobile phase most frequently used on Pirkle type stationary phases is 2-propanol.[13-16] Our results suggest that, a combination of suitable type of modifier, its concentration, and chemical structure obtained during derivatization of enantiomers can give the best results.

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