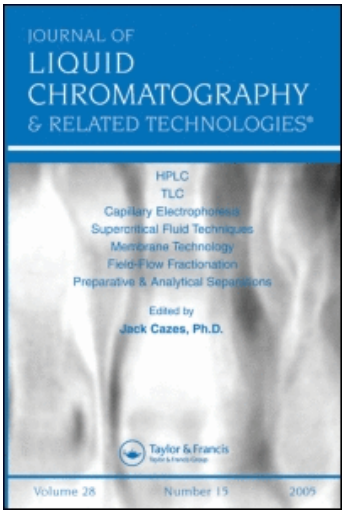

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Direct Separation of Enantiomers by High Performance Ligand Exchange Chromatography on Chemically Bonded Chiral Phases

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DIRECT SEPARATION OF ENANTIOMERS BY HIGH PERFORMANCE LIGAND EXCHANGE CHROMATOGRAPHY ON CHEMICALLY BONDED CHIRAL PHASES

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ABSTRACT

The application of chemically bonded chiral phases, prepared by binding various chelating ligands to silica gel via 3-glycidoxipropyltrimethoxysilane as spacer, for the separation of enantiomers, is described.

The influence of the nature of the matrix, the fixed ligand, the metal ion and the mobile phase on the enantioselectivity is discussed. Examples are given for the application of the system to the resolution of amino acids, amino acid derivatives, dipeptides and hydroxy acids.

INTRODUCTION

The principle of chiral ligand exchange chromatography (CLEC) was first used by Davankov for the separation of amino acid racemates[1-3]. This basic approach has subsequently been modified by several research groups[4]. In general, two main trends have

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developed. The first approach includes the application of sorbents containing chelating ligands fixed on them. The second approach is based on the use of chiral chelating agents as additives to the mobile phase. The first sorbents consisted of polymers like polystyrene[1,2] or polyacrylamide[5] as matrix containing chiral chelating ligands like amino acids. Loaded with metal ions these sorbents showed a remarkable enantioselectivity for amino acids. These polymers however, were not stable to pressure and not applicable to HPLC.

To combine the enantioselectivity of LEC with the efficiency of HPLC it was reasonable to develope phases on the basis of chemically modified silica gel. Guided by this approach we synthesized chiral phases by bonding L-amino acids and other chiral chelating ligands to silica gel using 3-glycidoxipropyltrimethoxysilane as a spacer. These phases were found to be highly enantioselective for amino acids[6-9].

Subsequently a series of further LEC-phases on the basis of silica gel have been developed by several working groups[4,10-18].

In this paper a view of the application fields of the different phases synthesized in our working group is given. The resolution of amino acids, amino acid derivatives, dipeptides and hydroxy acids is described.

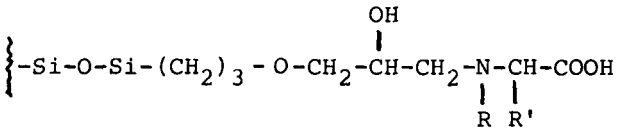
EXPERIMENTAL

Apparatus

A Perkin-Elmer liquid chromatograph series 2 equipped with a Rheodyne 7105 injector and a Perkin-Elmer LC 55 UV-detector was used. Polarimetric detection was carried out with a Perkin-Elmer polarimeter 241 MC with a micro flow-cell of 100 mm path length and 0.65 mm i.D. with a volume of 33 µl.

Chemicals and materials

The synthesis of the chiral phases of the general type I was carried out as previously described[7,9].



I

The phases were packed as a methanol slurry into stainless steel columns of 5 or 25 cm in length and 0.46 cm I.D. For the separations on a preparative scale 25 x 1.6 cm columns were used.

Mobile phases

10^{-5} M Cu(II)SO_4.

0.05 M KH_2PO_4, pH 4.6, containing 0.1 mmol Cu(II)SO_4/l.

0.05 M CH_3COONH_4/NH_3 of different pH, containing 0.1 mmol copper(II)acetate/l in different ratios with acetonitril.

RESULTS AND DISCUSSION

Amino acids:

The mechanismn of the resolution of amino acids by LEC has been widely discussed by Davankov[3,4,19]. The retention, enantioselectivity and elution order depends strongly on the nature of the matrix, the fixed ligand and the applied metal ion. Besides complex formation additional binding forces as hydrogene bondings to the hydroxy group in the side chain and residual hydroxy groups of the silica gel or hydrophobic interactions have to be taken into account. A possible structure of the mixed complex between proline as a fixed ligand and a D- or L-amino acid is presented in Fig. 1.

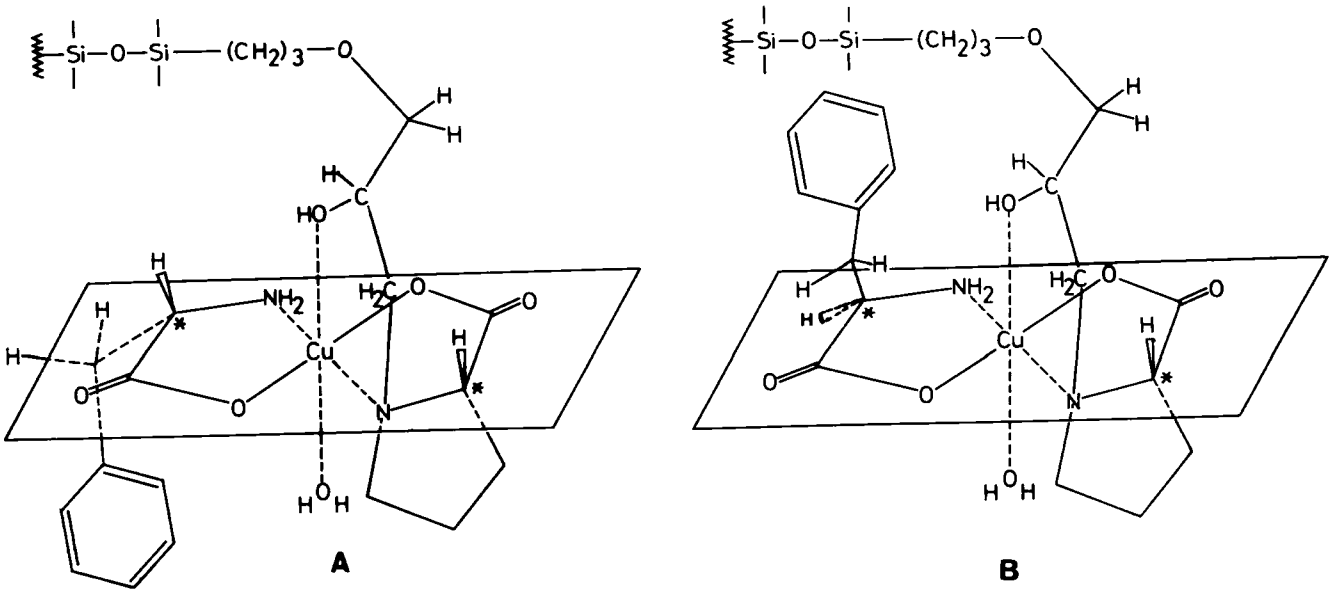


Figure 1

Model of the structure of the mixed ligand copper complex.

A: L-Proline-Cu-L-phenylalanine; B: L-proline-Cu-D-phenylalanine

Sterical hindrance between the substituent R in the D-form and the spacer group may lead to a weaker complexation compared to the L-form, where a free rotation is possible. This assumption explains the elution of the D-form before the L-form. The same elution order was observed on chiral phases based on polyacrylamide[5] and methacrylate polymers[20].

The reversed elution order for D,L-proline can be interpreted by the lack of the ability of the L-form to rotate because of the cyclic structure.

On the phenylalanine phase all amino acids show a reversed elution order. Probably steric reasons and hydrophobic interactions are responsible for this phenomenon. This hypothesis is an agreement with the fact, that on hydrophobic sorbents[2,4,15] the elution order L before D has been observed too.

The surface coverage was calculated from the results of the elemental analysis and was found to be 2 $\mu mol/m^2$ on average for the different phases. The number of theoretical plates is 2500 - 3000/m.

The compounds used as fixed ligands are listed in Table I. Cyclic amino acids as fixed ligands show a higher enantioselectivity than aliphatic ones. Among the cyclic amino acids the enantioselectivity for amino acids increases in the sequence azetidine carboxylic acid < proline < hydroxyproline < pipecolic acid. The hydroxyproline phase shows a high enantioselectivity especially for amino acids containing polar groups, probably due to the formation of additional hydrogene bondings.

Among the tested metal ions, Cu(II) was the most suitable one for the resolution of amino acids.

The composition and pH of the mobile phase also plays an important role in the retention behavior and enantioselectivity of amino acids. Water, potassiumdi-

TABLE I

Ligands fixed on the stationary Phase

L-Proline	L-Azetidine carboxylic acid
L-Hydroxyproline	L-Pipecolic acid
L-Valine	L-Propylendiamine
L-Histidine	L-Ephedrine
L-Phenylalanine	L-Tartaric acid

hydrogenephosphate and ammonacetate buffers of different pH with and without acetonitril were used as mobile phases. For stabilisation reasons small amounts of copper were added to the mobile phases.

All common amino acids could be resolved. The α-values of some examples of amino acids on different phases are listed in Table II. The separation of a mixture of amino acid racemates is shown in Fig. 2. Fig. 3 shows the resolution of α-methyl-DOPA, a compound of pharmaceutical interest. Only the L-form has an antihypertonic effect. An α-value of 8 is obtained.

Thyroid hormones (tetra-, tri-, and dijodothyronine) could be resolved on a 5 cm proline column by using ammonacetate buffer pH 8.5/ acetonitril 65:35 as mobile phase. Contrary to the L-enantiomer the D-form shows no basic metabolic rate enhancement, but a marked cholesterol level reducing effect.

The separation of amino acid racemates on a preparative scale was also investigated. On a 25 x 1.6 cm proline column with pure water as mobile phase, 50 mg phenylalanine could be separated.

TABLE II

Comparison of the K' and α-Values (K'_L/K'_D) for some Amino acid Enantiomers on Sorbents containing cylic Amino acids as stationary Ligands
Contitions as in Fig. 2

	Si-Pro			Si-Hypro			Si-Pip		
AA	K'_D	K'_L	α	K'_D	K'_L	α	K'_D	K'_L	α
Ala	1.30	1.30	1.00	1.40	1.40	1.00	1.40	1.25	0.89
Val	2.50	3.80	1.50	2.20	2.60	1.20	1.40	2.68	1.91
Leu	3.10	3.10	1.00	3.40	2.90	0.85	2.08	2.60	1,25
Ileu	3.00	3.60	1.20	3.40	3.40	1.00	1.70	2.75	1.62
Ser	2.00	3.20	1.60	2.20	3.40	1.55	1.63	2.83	1.74
Met	3.00	3.40	1.10	3.90	3.90	1.00	2.52	3.35	1.33
Lys	2.00	2.20	1.10	1.60	1.60	1.00	1.10	1.55	1.41
Arg	2.40	3.00	1.20	2.40	2.40	1.00	1.55	2.30	1.48
His	6.70	12.10	1.80	7.60	17.60	2.32	5.08	7.93	1.56
Asp	3.20	4.10	1.30	5.90	7.20	1.22	2.52	3.13	1.24
Glu	2.00	2.00	1.00	4.00	4.00	1.00	1.85	2.30	1.24
Pro	2.40	1.40	0.60	3.10	1.30	0.42	1.55	1.18	0.76
Phe	3.20	9.40	2.90	5.20	12.20	2.35	4.03	8.99	2.29
p-Tyr	3.30	10.20	3.10	3.60	15.00	4.17	4.33	14.45	3.34
DOPA	3.40	11.20	3.20	4.40	19.20	4.36	4.48	16.63	3.71
Trp	7.80	27.40	3.50	9.20	39.80	4.33	12.20	31.10	2.55

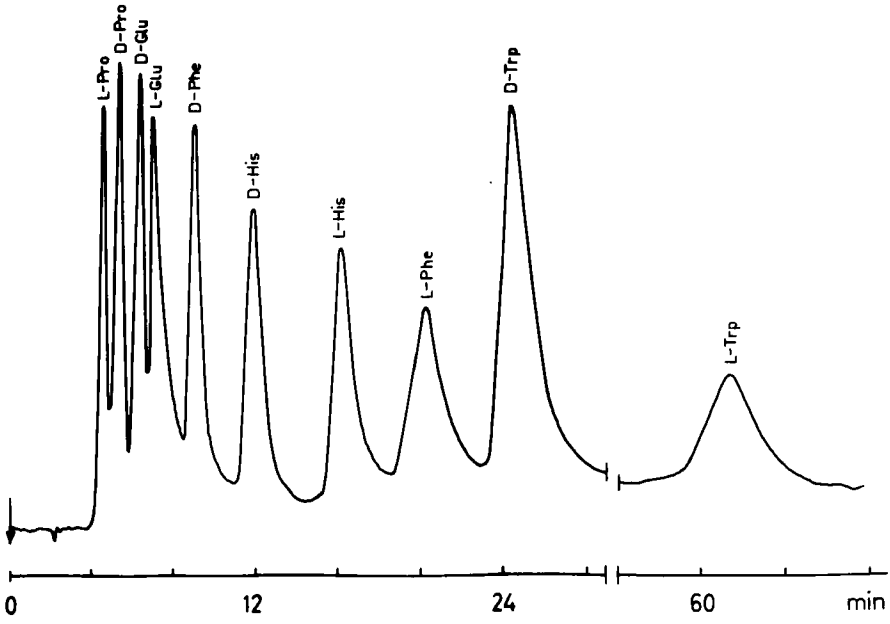


Figure 2

Resolution of a mixture of DL-amino acids.

Column: Si-Pip
Mobile phase: 0.05 M KH_2PO_4, pH 4.6, containing 0.1 mmol Cu(II)sulfate/l
Temp.: 50°C
Flow rate: 2 ml/min
Det.: UV 223 nm

Amino acid derivatives:

The method was found to be applicable to the resolution of dansyl-D,L-amino acids too. Taking an ammonacetate buffer pH 8/acetonitril as mobile phase a series of dansyl-D,L-amino acids could be resolved on the azetidine carboxylic acid phase.

Attempts to apply the method to esters of amino acids were not successful with the exception of D,L-

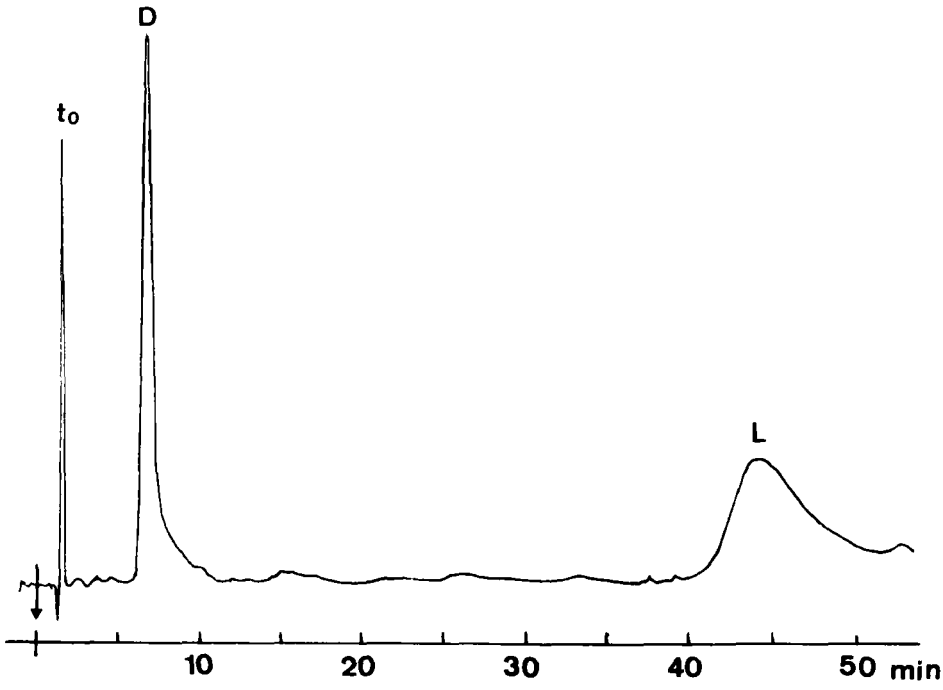


Figure 3

Resolution of α-methyl-DOPA

Column: Si-Hypro
Mobile phase: 0.05 M KH_2PO_4, pH 4.6, containing 0.1 mmol Cu(II)sulfate/l
Temp.: 50°C
Flow rate: 2 ml/min
Det.: UV 254 nm

histine methylester (Fig. 4). The ester group cannot take part in complex formation. In the case of histidine methylester a participation of the imidazolyl ring in the complex formation can be assumed.

Dipeptides:

Glycyl peptides could be separated in the same way as amino acids but water was used instead of buffers

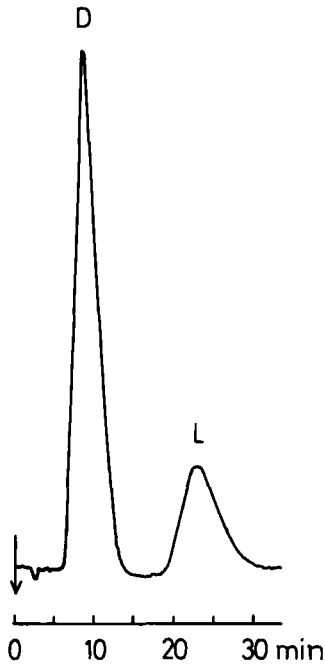


Figure 4

Resolution of histidine methylester
Column: Si-Pro
Mobile phase: 0.05 M KH_2PO_4, pH 4.6, containing 0.1 mmol Cu(II)sulfate/l
Temp.: 50°C
Flow rate: 2 ml/min
Det.: UV 223 nm

as mobile phase. The highest enantioselectivity for dipeptides was observed on the hydroxyproline- and the azetidine carboxylic acid phase. In Table III the K'- and α-values of some glycyl peptides are given. The resolution of a mixture of 4 glycyl peptides is shown in Fig. 5.

As preliminary experiments proved, diastereomeric dipeptides can also be resolved. For D,L-leucyl-D,L-

TABLE III

K' and α-Values of Glycyl-dipeptide Enantiomers
Conditions as in Fig. 5

	K_L	K_D	α
Glycyl-alanine	1.5	1.13	1.33
Glycyl-valine	4.25	2.63	1.62
Glycyl-norvaline	3.00	1.94	1.55
Glycyl-leucine	5.00	3.00	1.67
Glycyl-norleucine	3.13	2.00	1.57
Glycyl-serine	1.00	0.75	1.33
Glycyl-threonine	2.00	1.25	1.60
Glycyl-methionine	2.19	1.44	1.52
Glycyl-asparagine	1.25	0.94	1.33
Glycyl-phenylalanine	4.00	1.50	2.67
Glycyl-tryptophan	8.13	2.19	3.71

TABLE IV

K' and α-Values of Hydroxy acid Enantiomers
Conditions as in Fig. 7

DL-Hydroxy acid	$K_{(+)}$	$K_{(-)}$	$\alpha=\frac{K(-)}{K(+)}$
Lactic acid	0.68	0.97	1.42
Mandelic acid	1.01	1.67	1.65
3-Hydroxymandelic acid	1.00	1.62	1.62
4-Hydroxymandelic acid	0.90	1,59	1,76
3,4-Dihydroxymandelic acid	1.57	2.00	1.27
3-Phenyllactic acid	3.28	5.28	1.61
Atrolactic acid (2-Phenyllactic ac.)	1.02	1.49	1.46
2-Hydroxycaproic acid	1.88	3.42	1.82
Tropic acid	2.00	2.75	1.31

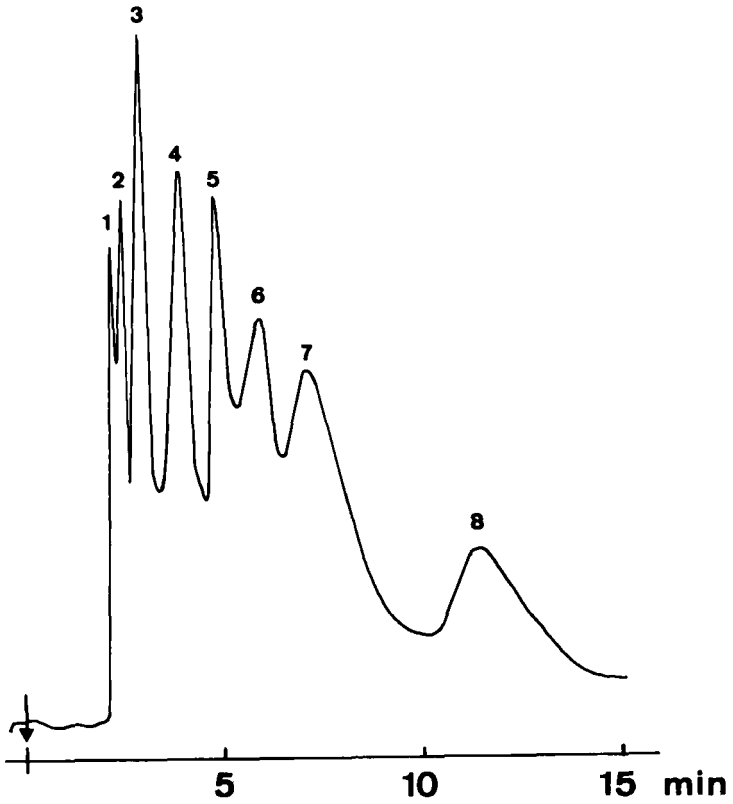


Figure 5

Separation of glycyl-DL-dipeptides

Column: Si-Hypro
Mobile phase: 10^{-5}M Cu(II)SO_4
Temp.: 20°C
Flow rate: 1 ml/min
Det.: UV 223 nm

1: Gly-D-ser; 2: gly-L-ser; 3: gly-D-phe; 4: gly-D-trp; 5: gly-D-leu; 6: gly-L-phe; 7: gly-L-leu; 8: gly-L-trp.

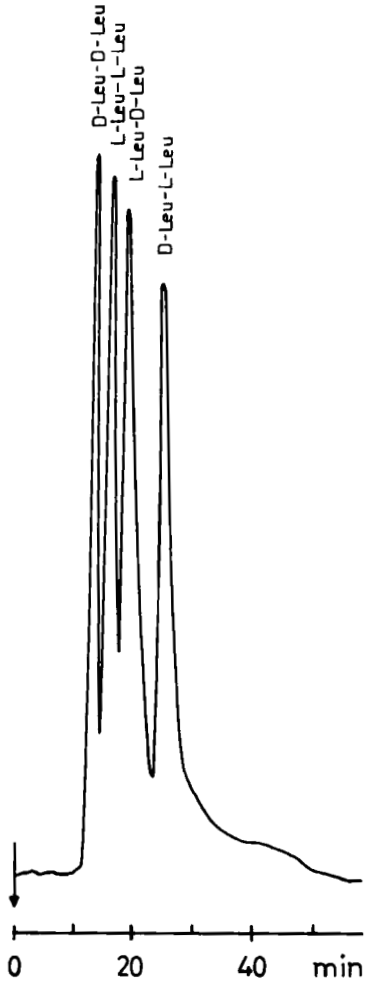


Figure 6

Resolution of DL-leucyl-DL-leucine
Conditions as in Fig. 5

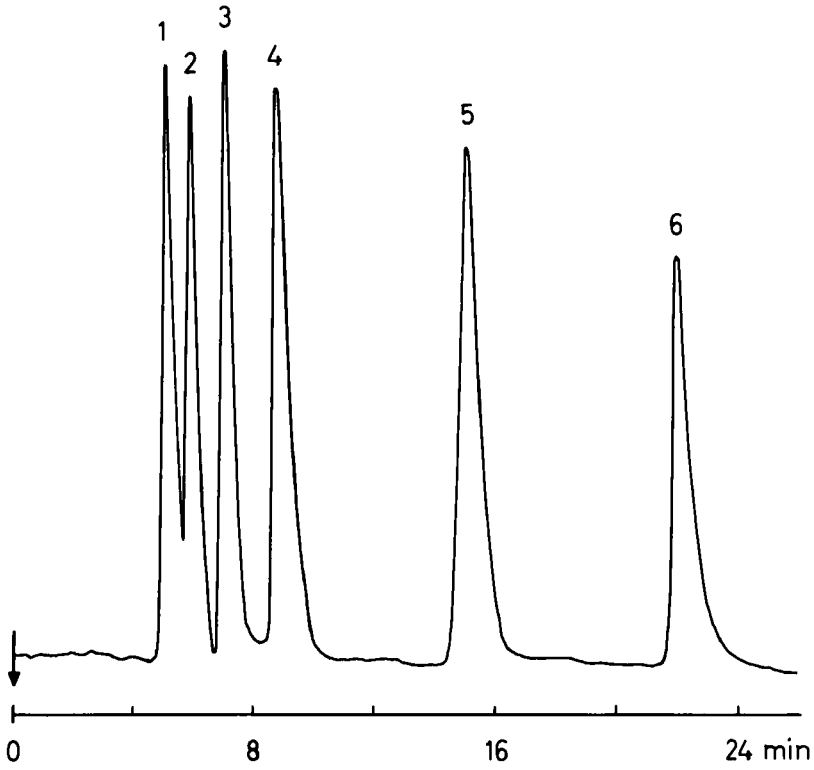


Figure 7

Separation of hydroxy acid enantiomers

Column: Si-Hypro$_{-5}$
Mobile phase: 10^{-5}M Cu(II)SO_4
Temp.: 20°C
Flow rate: 2 ml/min
Det.: UV 223

1: D(+)-Lactic acid; 2: L(-)-lactic acid; 3: D(+)-atrolactic acid; 4: L(-)-atrolactic acid; 5: D(+)-3-phenyllactic acid; 6: L(-)-3-phenyllactic acid.

leucine 4 peaks for the single enantiomers occured (Fig. 6). D,L-Leucyl-D,L-phenylalanine showed the same result. In the case of D,L-leucyl-D,L-thyrosine only 3 peaks were obtained. Not all enantiomers being available, an identification of the peaks was not possible. Further research on this subject will be done.

Hydroxy acids:

The hydroxyproline phase showed a marked enantioselectivity for α-hydroxy acids[21]. With a 10^{-4}M copper(II)sulfate as mobile phase a series of α-hydroxy acids could be resolved. In Table IV the K' and α-values of some examples of hydroxy acids are given. The formation of 5 membered chelate complexes is normally prefered. In the case of tropic acid, which is a β-hydroxy acid, the formation of a 6-membered ring can be assumed.

The resolution of a mixture of 3 hydroxy acid racemates is shown in Fig. 7.

CONCLUSIONS

This view demonstrates the applicability of the above presented chiral phases for the resolution of a broad spectrum of racemic compounds. Different fixed ligands showed distinct selectivity. α-values up to 8 have been observed. The possibility of the transfer of the system to preparative scale has been demonstrated. The application of the method for the resolution of racemic drugs is under investigation.

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