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THIN-LAYER CHROMATOGRAPHIC ENANTIOMERIC RESOLUTION VIA LIGAND EXCHANGE

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SUMMARY

A thin-layer chromatographic technique for the separation of proteinogenic and non-proteinogenic amino acids, dipeptides and α-hydroxy acids is described. Other examples are given from the field of α-methyl, N-alkyl and halogenated amino acids. The separation of the enantiomers is achieved, without derivatization, by means of ligand exchange on a reversed-phase silica gel as stationary phase, which is covered with a chiral selector (proline derivative). The resolution is so good that the respective enantiomers can be determined at trace levels ($\geqslant 0.25\%$). The proposed method is simple, inexpensive and needs no sophisticated instruments.

INTRODUCTION

The activity and effectiveness of chiral molecules depend largely on their configuration[1]. Often only one of the enantiomers is pharmacologically active, while the other may be at best inactive or even toxic. Only *ca.* 20% of the optically active pharmaceuticals are traded as pure enantiomers[2]. This has resulted in an increasing interest in stereoselective syntheses, and in the growing importance of optically active amino acids, which are commercially available on a large scale as a "chiral pool", as building blocks and auxiliaries for the production of biologically active peptides, in asymmetric syntheses and as pharmaceuticals.

This is the reason why efficient analytical procedures for the control of optical purity have to be developed as aids for asymmetric syntheses. Traditionally, many laboratories use polarimetry for the control of optical purity, but this method suffers from some well-known specific drawbacks. Additionally, the calculation of the enantiomeric excess (*ee*) from optical rotation may be impossible, because the specific rotation of the pure enantiomer is unknown, or calculated *ee* values may be wrong owing to impurities. Therefore direct, *e.g.* chromatographic, analytical procedures are to be preferred.

Because simple separation techniques were not available until recently, gas chromatographic[3–6] or liquid chromatographic[7–13] methods are generally used for direct determination of the enantiomeric composition. These systems need expensive instruments, are often "derivative", and, for routine application require that

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standardized stationary phases are commercially available. Therefore the application of thin-layer chromatographic (TLC) separation techniques, especially for large test series, is desirable. In addition, TLC allows easy control of a synthetic process by laboratory personnel.

With the introduction by Günther *et al.*[14] in 1983 of racemic separations by TLC based on ligand exchange, an alternative method is available that meets the requirements mentioned above and enables rapid determination of the enantiomeric distribution at trace levels. This paper describes in detail the results of this work. For the numerous and interesting papers dealing with paper chromatographic (PC) or TLC enantiomeric separations on adsorbents with chiral cavities (such as cellulose, cyclodextrin, triacetylcellulose), the reader is referred to the following literature: PC, refs. 15–34; TLC, refs. 35–46. Owing to extremely long developing times[44]these layers, with the exception of triacetylcellulose[45], today find only limited application for routine control of the optical purity of chiral compounds.

This review covers procedures for the separation of underivatized samples on chiral stationary phases. Derivatization procedures[47,48] via ligand exchange are not discussed.

TLC ENANTIOMERIC SEPARATION VIA LIGAND EXCHANGE

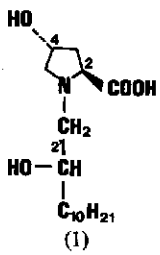
Mechanism of separation

Recent experimental results have confirmed the principle of chiral interaction (three-point-rule) postulated as early as 1952 by Dalgliesh[19]. Additionally the results prove that the separation models developed for ligand exchange by high-performance liquid chromatography (HPLC)[11,49,50] are also valid for TLC: the diastereomeric complexes formed with the metal ion (*e.g.* Cu^{2+}) and the chiral adsorbent have different stabilities for the different antipodes, and thus chromatographic separation is achieved.

Separation of racemates on reversed-phase layers

Because, like others, our experiments to reduce the development times on cellulose by variation of the eluent composition failed, the commercial availability of reversed-phase (RP) precoated plates offered a good opportunity to study the racemic separation of amino acids via ligand exchange on these layers.

Based on the work of Davankov *et al.*[10,49], who modified commercial HPLC columns for distribution chromatography with alkyl derivatives of L-amino acids, such as *n*-decyl-L-histidine or *n*-hexadecyl-L-proline, we used (2*S*,4*R*,2′*RS*)-N-(2′-hydroxydodecyl)-4-hydroxyproline (1)[51], which was easier to prepare, as a chiral selector.



(1)

Preparation of an enantioselective layer. The following impregnation procedure proved to be most efficient. A glass plate coated with hydrophobic silica gel (RP 18 TLC) was dipped into a 0.25% copper(II) acetate solution (methanol–water, 1:9, v/v) and dried. Then the plate was immersed in a 0.8% methanolic solution of the chiral selector for 1 min. After air-drying, the plate was ready for enantiomeric separations. The first results of this work were published in 1984[52].

Further development of the plate has been made in collaboration with Macherey-Nagel (Düren, F.R.G.). The plate is now commercially available as a precoated plate under the trade name Chiralplate: preparatory dipping is no longer required.

Selection of mobile phase and influence of mobile phase composition on separation of enantiomers. The choice of eluents for the separation experiments described here was based on experience with HPLC mobile phases. In order to achieve short analysis times, ternary mixtures of a water-miscible alcohol, water and acetonitrile proved useful. Most racemate separations could be accomplished using one of two eluent systems: eluent A, methanol–water–acetonitrile (50:50:200, v/v/v), development time *ca.* 30 min; eluent B, methanol–water–acetonitrile (50:50:30, v/v/v), development time *ca.* 60 min. As expected, a decrease in the acetonitrile content significantly increased the development times.

For some substances, however, different eluent systems were more suitable: eluent C, methanol–water (10:80, v/v), development time *ca.* 90 min, for leucine; eluent D, acetone–methanol–water (10:2:2, v/v/v), development time *ca.* 50 min, for alanine and serine; eluent E, dichloromethane–methanol (45:5, v/v), development time *ca.* 20 min, for α-hydroxy acids.

Brinkman and Kamminga[53] systematically investigated the influence of binary mixtures on the enantiomeric separation of different amino acids. With TLC precoated plates (Chiralplate) of size 4 cm × 6 cm, they separated the enantiomers in 4 min in water–acetonitrile (20:80).

Chromatographic conditions. The best results on the precoated plate (Chiralplate) were obtained with the following conditions. Method: ascending, one-dimensional development in a TLC chamber with chamber saturation. Plates: TLC precoated Chiralplates (Cat. No. 811055/056, Macherey-Nagel); size, 10 cm × 20 cm; layer thickness, 0.25 mm. With eluents A, B, and C, 2 μl of a 1%solution of the racemate (methanol or methanol–water) were applied. With eluent D, 2 μl of a 0.5% solution of the racemate [0.1 *M* hydrochloric acid–methanol (1:1, v/v)] were applied. With eluent E, 2 μl of a 0.5% solution of the racemate (methanol) were applied. The migration distance was 13 cm.

Different detection methods were used, depending on the type of compound. For proteinogenic and non-proteinogenic amino acids, the dried plates were dipped for 3 s in a 0.3% ninhydrin solution in acetone (Tauchfix, Baron) and then dried in a drying cabinet for *ca.* 5 min at 110°C. Red derivatives formed on a white background. For α-hydroxycarboxylic acids, 1.82 g of vanadium pentoxide (Merck, Art. 824) were weighed into a 100-ml measuring flask, 30 ml of 1 *M* sodium carbonate were added and completely dissolved by treatment in an ultrasonic bath. After cooling, 46 ml of 2.5 *M* sulphuric acid and acetonitrile to 100 ml were added. The dried plates were briefly (set 2 s on the Tauchfix) dipped into this solution and then left to stand at room temperature for *ca.* 45 min. Blue derivatives formed on a yellow background.

SELECTED EXAMPLES OF SEPARATION

With the technique described, more than 100 racemate separations have been accomplished by our group, most of which have been published[52,54–61]. For this reason we shall not describe all the examples in this paper, but rather demonstrate the broad range of application of this method for some selected classes of compounds, such as amino acid and peptide analysis; the enantiomeric separation of α-hydroxycarboxylic acids will be discussed in detail.

Amino acids

Thus far, twelve proteinogenic amino acids have been separated without

TABLE I

TLC SEPARATION OF PROTEINOGENIC AND NON-PROTEINOGENIC AMINO ACIDS*

Racemate	R_F *value*	*(Configuration)*	*Eluent***
Alanine	0.69 (D)	0.73 (L)	D
Aspartic acid	0.50 (D)	0.55 (L)	A
Glutamic acid	0.54 (D)	0.59 (L)	A
Glutamine	0.41 (L)	0.55 (D)	A
Isoleucine	0.47 (D)	0.58 (L)	A
Leucine	0.53 (D)	0.63 (L)	C
Methionine	0.54 (D)	0.59 (L)	A
Valine	0.54 (D)	0.62 (L)	A
Phenylalanine	0.49 (D)	0.59 (L)	A
Serine	0.73 (D)	0.76 (L)	D
Tyrosine	0.58 (D)	0.66 (L)	A
Tryptophan	0.51 (D)	0.61 (L)	A
Proline	0.41 (D)	0.47 (L)	A
Cysteine (as thiazolidine-4-carboxylic acid)	0.59 (D)	0.69 (L)	A
tert.-Leucine	0.40 (D)	0.51 (L)	A
Norleucine	0.53 (D)	0.62 (L)	A
allo-Isoleucine	0.51 (D)	0.61 (L)	A
Norvaline	0.49 (D)	0.56 (L)	A
allo-4-Hydroxyproline	0.41 (L)	0.59 (D)	A
2-Phenylglycine	0.57 (D)	0.67 (L)	A
2-Cyclopentylglycine	0.43	0.50	A
Ethionine	0.52 (D)	0.59 (L)	A
(1-Naphthyl)-alanine	0.49 (D)	0.56 (L)	A
(2-Naphthyl)-alanine	0.44 (D)	0.59 (L)	A
O-Benzylserine	0.54 (D)	0.65 (L)	A
O-Benzyltyrosine	0.48 (D)	0.64 (L)	A
4-Methyltryptophan	0.50	0.58	A
4-Methoxyphenylalanine	0.52	0.64	A
5-Methoxytryptophan	0.55	0.66	A
Methioninesulphone	0.62 (D)	0.66 (L)	A
Ethioninesulphone	0.55	0.59	A
Selenomethionine	0.53 (D)	0.61 (L)	A
Dopa	0.47 (L)	0.58 (D)	B

* Migration distance 13 cm; chamber saturation.

** (A) Methanol–water–acetonitrile, 50:50:200 (v/v/v); (B) methanol–water–acetonitrile, 50:50:30 (v/v/v); (C) methanol–water, 10:80 (v/v); (D) acetone–methanol–water, 10:2:2 (v/v/v).

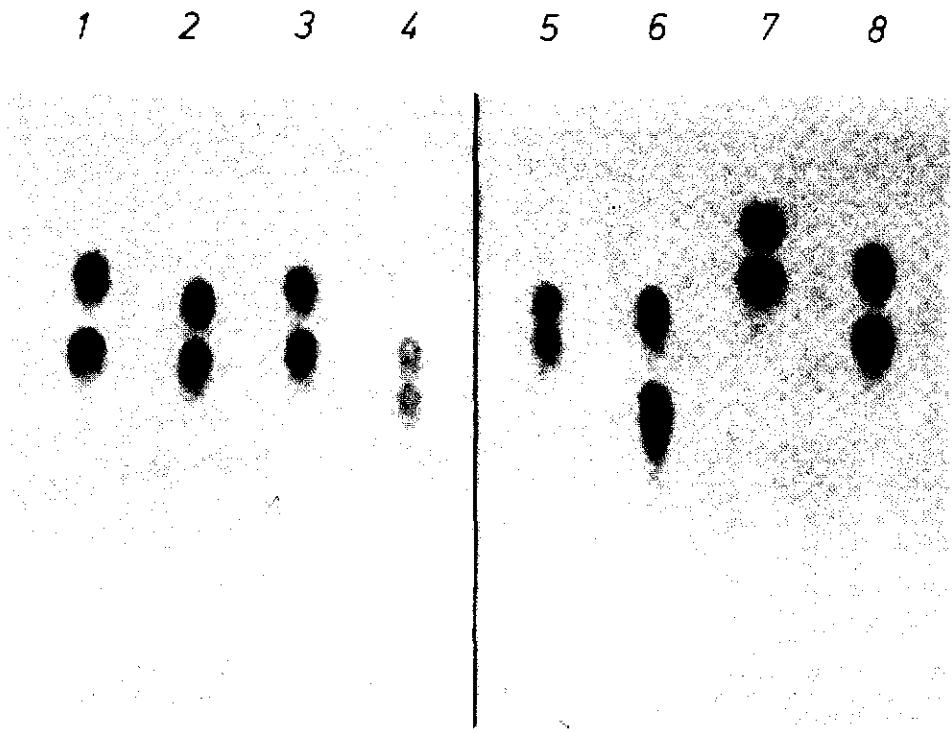


Fig. 1. Photograph of a thin-layer chromatogram of proteinogenic amino acids on Chiralplate. Spots: 1 = phenylalanine; 2 = valine; 3 = isoleucine; 4 = proline; 5 = methionine; 6 = glutamine; 7 = tyrosine; 8 = tryptophan.

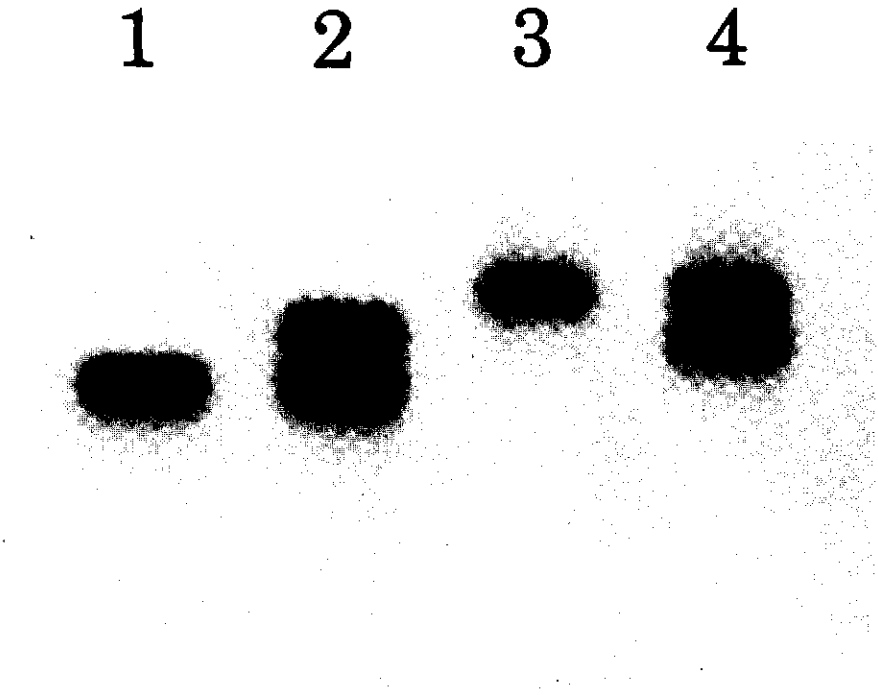


Fig. 2. Photograph of a thin-layer chromatogram of proteinogenic amino acids on Chiralplate. Spots: 1 = D-alanine; 2 = D,L-alanine; 3 = L-serine; 4 = D,L-serine. Application: 10 mm streak (Linomat IV, Fa. Camag).

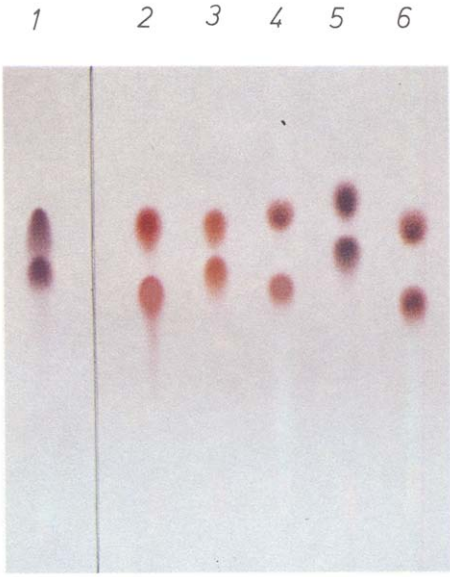


Fig. 3. Photograph of a thin-layer chromatogram of non-proteinogenic amino acids on Chiralplate. Spots: 1 = dopa; 2 = *tert.*-leucine; 3 = *allo*-isoleucine; 4 = O-benzyltyrosine; 5 = 5-methoxytryptophan; 6 = (2-naphthyl)alanine.

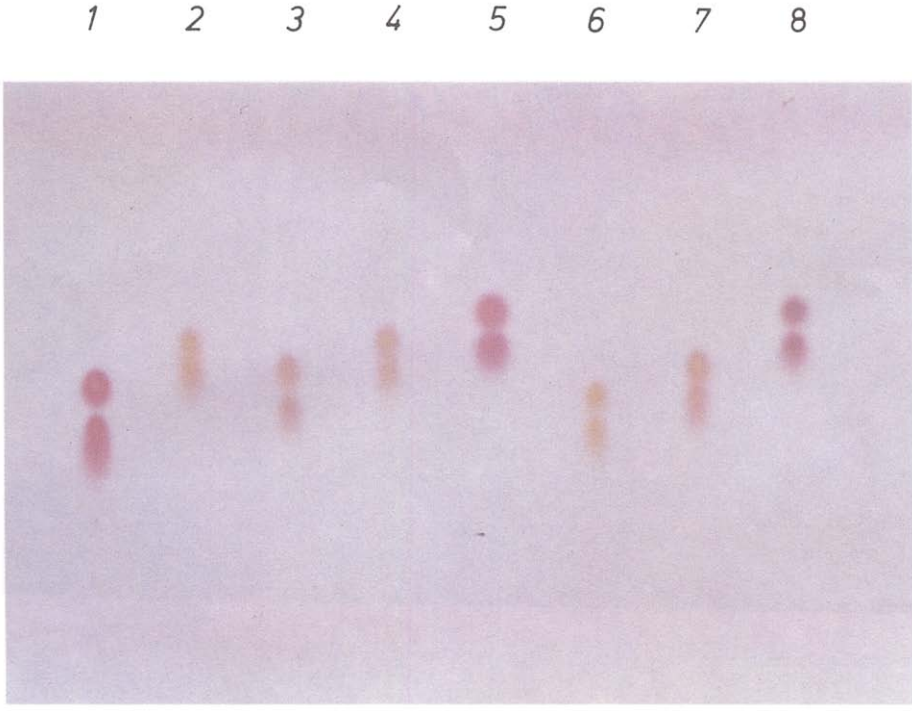


Fig. 4. Photograph of a thin-layer chromatogram of dipeptides on Chiralplate. Spots: 1 = D-Leu-L-Leu and L-Leu-D-Leu; 2 = Gly-D,L-Val; 3 = Gly-D,L-Leu; 4 = Gly-D,L-Phe; 5 = D-Ala-L-Phe and L-Ala-D-Phe; 6 = Gly-D,L-Trp; 7 = Gly-D,L-Ileu; 8 = D-Met-L-Met and L-Met-D-Met.

TABLE II

TLC ENANTIOMERIC SEPARATION OF DIPEPTIDES*

Dipeptide	R_F *value*	*(Configuration)*	*Eluent***
Gly-D,L-Phe	0.57(L)	0.63(D)	B
Gly-D,L-Leu	0.53(L)	0.60(D)	B
Gly-D,L-Ileu	0.54(L)	0.61(D)	B
Gly-D,L-Val	0.58(L)	0.62(D)	B
Gly-D,L-Trp	0.48(L)	0.55(D)	B
D-Leu-L-Leu	0.48		B
L-Leu-D-Leu	0.57		B
D-Leu-L-Leu	0.19		A
L-Leu-D-Leu	0.26		A
D-Ala-L-Phe	0.59		B
L-Ala-D-Phe	0.65		B
D-Ala-L-Phe	0.21		A
L-Ala-D-Phe	0.26		A
D-Met-L-Met	0.64		B
L-Met-D-Met	0.71		B
D-Met-L-Met	0.29		A
L-Met-D-Met	0.33		A

* Migration distance 13 cm, chamber saturation.

** (A) Methanol–water–acetonitrile, 50:50:200 (v/v/v); (B) methanol–water–acetonitrile, 50:50:30 (v/v/v).

derivatization (Table I; Figs. 1 and 2); cysteine can be determined as thiazolidine-4-carboxylic acid, which is formed from cysteine by a simple reaction with formaldehyde.

The separation of non-proteinogenic amino acids is shown in Fig. 3.

Dipeptides

For the enantiomeric separation of dipeptides (see Table II) it is remarkable that the enantiomer with the C-terminal L-configuration always has lower R_F value than the one with the C-terminal D-configuration (see Fig. 4). This method can also resolve diastereomeric dipeptides as well[57].

TABLE III

TLC SEPARATION OF ENANTIOMERIC α-METHYLAMINO ACIDS*

Racemate	R_F *value*	*(Configuration)*	*Eluent***
α-Methylmethionine	0.56(D)	0.64(D)	A
α-Methylserine	0.56(L)	0.67(D)	B
α-Methyltyrosine	0.63(D)	0.70(L)	A
α-Methylphenylalanine	0.53(L)	0.66(D)	A
α-Methyldopa	0.46(L)	0.66(D)	B

* Migration distance 13 cm; chamber saturation.

** (A) Methanol–water–acetonitrile, 50:50:200 (v/v/v); (B) methanol–water–acetonitrile, 50:50:30 (v/v/v).

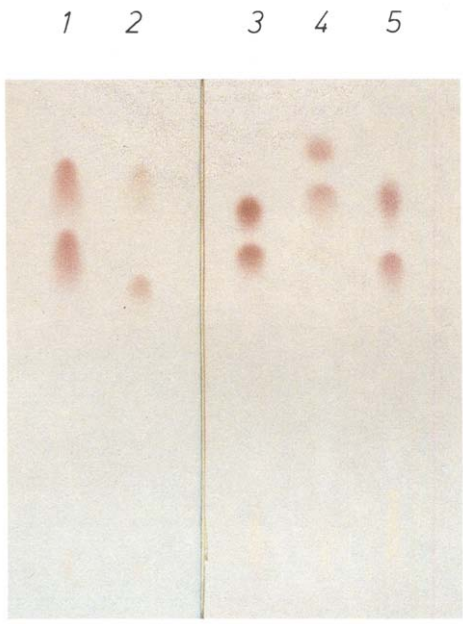


Fig. 5. Photograph of a thin-layer chromatogram of α-methylamino acids on Chiralplate. Spots: 1 = α-methylserine; 2 = α-methyldopa; 3 = α-methylmethionine; 4 = α-methyltyrosine; 5 = α-methylphenylalanine.

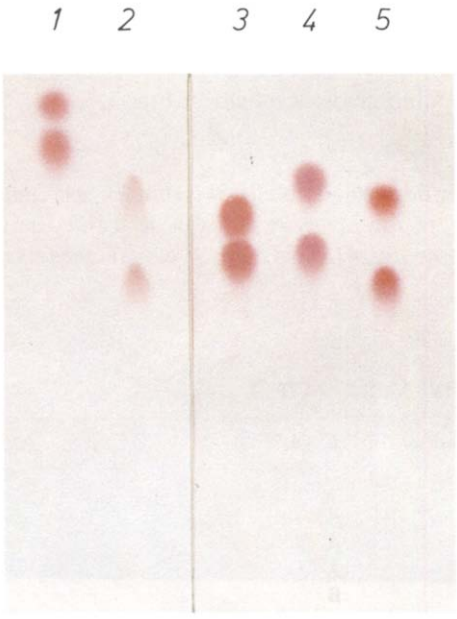


Fig. 6. Photograph of a thin-layer chromatogram of N-methyl and N-formyl amino acids on Chiralplate. Spots: 1 = N-methylvaline; 2 = N-methyl-*m*-tyrosine; 3 = N-methylleucine; 4 = N-methylphenylalanine; 5 = N-formyl-*tert.*-leucine.

TABLE IV

TLC SEPARATION OF ENANTIOMERIC N-ALKYL AND N-FORMYL AMINO ACIDS*

Racemate	R_F *value*	*(Configuration)*	*Eluent***
N-Methylleucine	0.49(L)	0.57(D)	A
N-Methylvaline	0.65(L)	0.70(D)	B
N-Methylphenylalanine	0.50(D)	0.61(L)	A
N-Methyl-*m*-tyrosine	0.36	0.52	B
N,N-Dimethylphenylalanine	0.55(D)	0.61(L)	B
N-Formyl-*tert.*-leucine	0.48 (+)	0.61 (−)	A

* Migration distance 13 cm.

** (A) Methanol–water–acetonitrile, 50:50:200 (v/v/v); (B) methanol–water–acetonitrile, 50:50:30 (v/v/v).

TABLE V

TLC SEPARATION OF HALOGENATED AMINO ACIDS*

Racemate	R_F *value*	*(Configuration)*
3-Chloroalanine	0.57	0.64
4-Bromophenylalanine	0.44	0.58
4-Chlorophenylalanine	0.46	0.59
2-Fluorophenylalanine	0.55	0.61
4-Iodophenylalanine	0.45(D)	0.61(L)
3-Fluorotyrosine	0.64	0.71
5-Bromotryptophan	0.46	0.58
Thyroxine	0.38(D)	0.49(L)

* Migration distance 13 cm; chamber saturation; eluent, methanol–water–acetonitrile, 50:50:200 (v/v/v).

TABLE VI

TLC ENANTIOMERIC SEPARATION OF OTHER CLASSES OF COMPOUNDS*

Racemate	R_F *value*	*(Configuration)*	*Eluent***
Thiazolidine-4-carboxylic acid	0.59(D)	0.69(L)	A
5,5-Dimethylthiazolidine-4-carboxylic acid hydrochloride	0.48 (D)	0.62(L)	A
3-Amino-3,5,5-trimethylbutyrolactone hydrochloride	0.50	0.59	A
Pipecolic acid	0.51	0.58	D

* Migration distance 13 cm; chamber saturation.

** (A) Methanol–water–acetonitrile, 50:50:200 (v/v/v); (D) acetone–methanol–water, 10:2:2 (v/v/v).

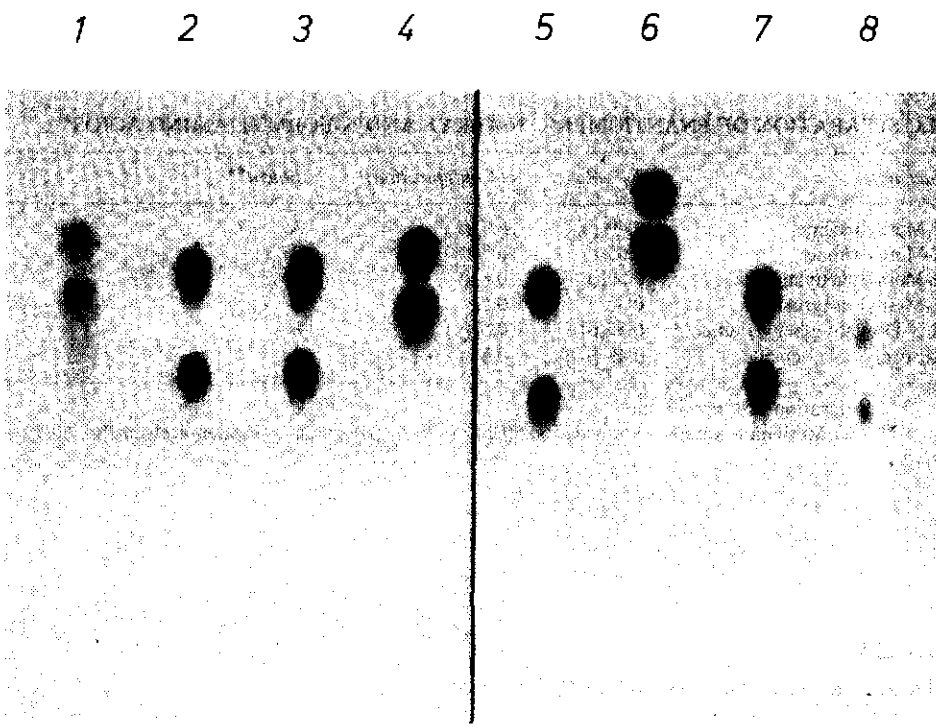


Fig. 7. Photograph of a thin-layer chromatogram of halogenated amino acids on Chiralplate. Spots: 1 = 3-chloroalanine; 2 = 4-bromophenylalanine; 3 = 4-chlorophenylalanine; 4 = 2-fluorophenylalanine; 5 = 4-iodophenylalanine; 6 = 3-fluorotyrosine; 7 = 5-bromotryptophan; 8 = thyroxine.

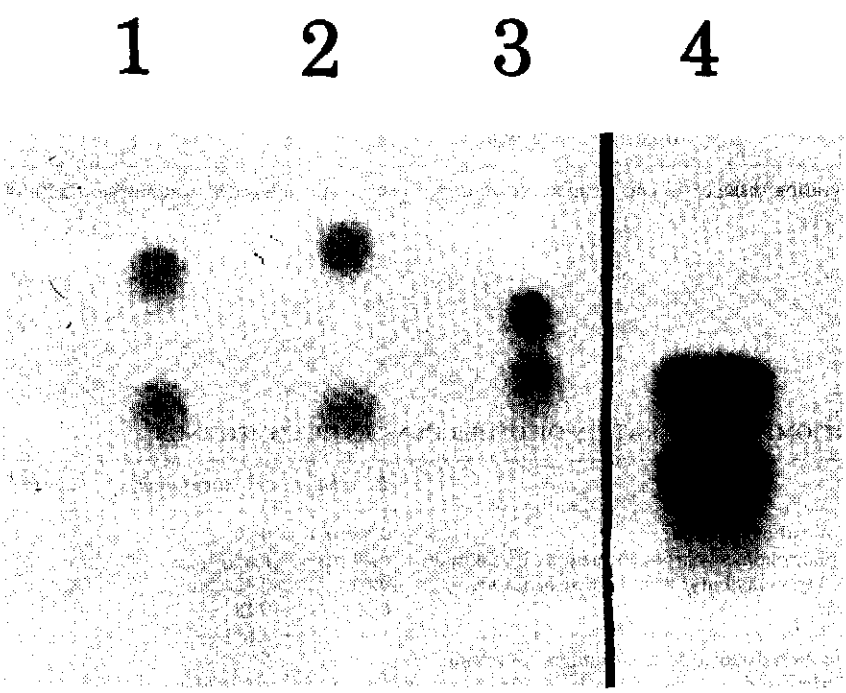


Fig. 8. Photograph of a thin-layer chromatogram of some heterocyclics on Chiralplate. Spots: 1 = thiazolidine-4-carboxylic acid; 2 = 5,5-dimethylthiazolidine-4-carboxylic acid; 3 = 3-amino-3,5,5-trimethylbutyrolactone hydrochloride; 4 = pipecolic acid.

Wang *et al.*[46] compared the migration and separation characteristics of dipeptides on Chiralplate with those on cellulose.

α-Methylamino acids

α-Methylamino acids are very important as specific enzyme inhibitors. Furthermore they can be directly inserted into numerous biologically active peptides to modify their range of activity.

Separations in this field with different eluent systems have been published independently (Fig. 5, refs. 60 and 62). As can be seen from Table III, D,L-methyldopa can also be separated without problem[59].

N-Alkylamino acids

Table IV and Fig. 6 show the separation of enantiomeric N-alkylamino acids and N-formyl-*tert.*-leucine. Further examples have been published recently[54,55]. In contrast to the examples described above, the detection of N,N-dimethylphenylalanine was achieved with iodine. The enantiomeric separation of N-carbamoyltryptophan has also been described[63].

Halogenated amino acids

Another class of compounds that show good enantiomeric resolution is the halogenated amino acids (Table V and Fig. 7). However, a differentiation between 4-chloro-, 4-bromo- and 4-iodophenylalanines is not possible[54,55].

Heterocyclic compounds

Thiazolidine-4-carboxylic acid and 5,5-dimethylthiazolidine-4-carboxylic acid are formed by formaldehyde condensation from cysteine and penicillamine, respectively. The derivatization of penicillamine has been published[58]. Table VI and Fig. 8 present an extract from these results.

α-Hydroxycarboxylic acids

During investigation of the enantioselective degradation of the biogenic

TABLE VII

TLC ENANTIOMERIC α-HYDROXYCARBOXYLIC ACIDS*

Racemate	R_F *value*	*(Configuration)*
Mandelic acid	0.46	0.53 (L)
3-Hydroxymandelic acid	0.34	0.39
4-Hydroxymandelic acid	0.32	0.36
3,4-Dihydroxymandelic acid	0.32	0.38
Vanillic acid	0.49	0.55
Hydroxyisoleucine	0.56	0.63(L)
Hydroxyleucine (sodium salt)	0.56	0.60(L)
Hydroxymethionine (sodium salt)	0.52	0.58(L)
Hydroxyphenylalanine	0.56	0.62(L)
Hydroxyvaline	0.52	0.60(L)

* Migration distance 13 cm; eluent, dichloromethane–methanol, 45:5 (v/v).

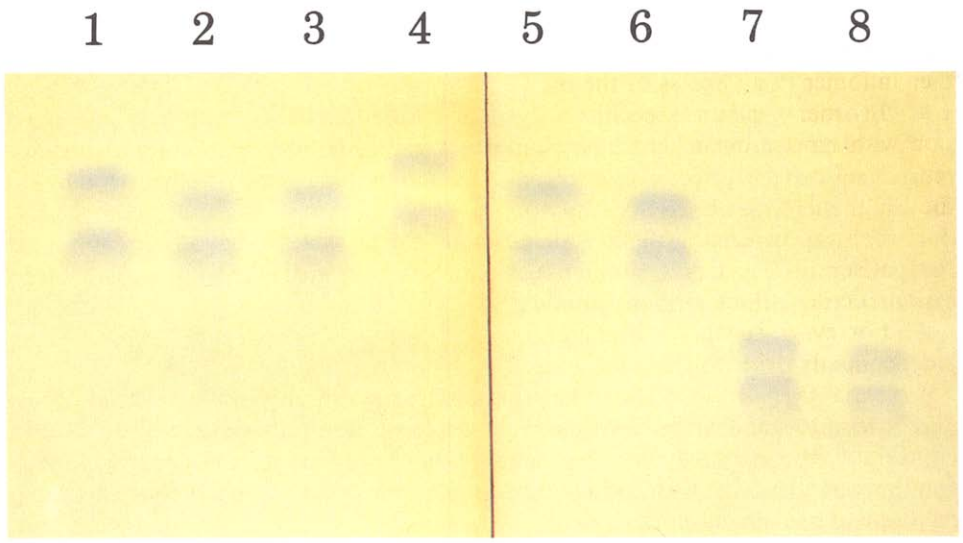


Fig. 9. Photograph of a thin-layer chromatogram of α-hydroxycarboxylic acids on Chiralplate. Spots: 1 = hydroxyisoleucine; 2 = hydroxyleucine; 3 = hydroxymethionine; 4 = hydroxyphenylalanine; 5 = hydroxyvaline; 6 = mandelic acid; 7 = 3-hydroxymandelic acid; 8 = 4-hydroxymandelic acid.

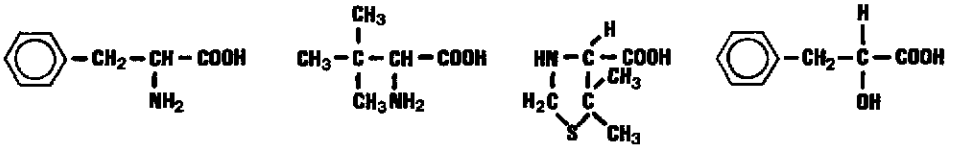
R-configurated catecholamines norepinephrine (noradrenaline) and epinephrine (adrenaline), Jork and Kany[64] for the first time succeeded in the enantiomeric separation of the resulting 3,4-dihydroxymandelic acid and vanillic acid, respectively, using the lipophilic eluent mixture dichloromethane–methanol (45:5, v/v) and post-chromatographic detection with 2,6-dichloroquinone-4-chloroimide (Merck, Art. 3037).

In preparative chemistry, mandelic acid is gaining importance as a multifunctional chiral building block and reagent for racemate separation. Table VII and Fig. 9 show the resolution of further enantiomeric α-hydroxycarboxylic acids. Vanadium pentoxide was especially useful for post-chromatographic derivatization[65].

QUANTITATIVE EVALUATION OF TLC-SEPARATED ENANTIOMERS

General

Phenylalanine (2), *tert.*-leucine (3), 5,5-dimethylthiazolidine-4-carboxylic acid (4) and hydroxyphenylalanine (5) have been chosen as models for the direct quantitative evaluation of thin-layer chromatograms.



2 3 4 5

Emphasis has been placed on the evaluation of detection limits for the TLC-separated enantiomers, because exact determination of trace levels of a D- or L-enantiomer in an excess of the other is increasingly important[61,66,67].

In order to enhance specificity and sensitivity, post-chromatographic derivatization with ninhydrin or vanadium pentoxide was used. Dipping the plates into the reagent solution proved most useful because it can be automated[68]. Quantification of the minor enantiomer was achieved by *in situ* remission measurement with the CS-930 double beam scanner (Shimadzu) or the densitometer CD 60 (Desaga), and comparison with external standard solutions. Additionally, possible proportional systematic deviations were excluded by the standard addition method[69].

For every substance investigated the absorption maximum was determined independently prior to the quantification experiments.

Preparation of test solutions and standard solutions

Successful separation of amino acids on the TLC plate inherently depends on the concentration and often on the hydrochloric acid content of the applied solution. Addition of hydrochloric acid generally improves the solubility of the amino acids and often considerably enhances the enantiomeric resolution.

Phenylalanine test solution (U_{Ph}). Weigh 200 mg of D-phenylalanine into a 10-ml measuring flask and fill to the mark with 50% methanolic hydrochloric acid solution (10 g of acid per litre of solution).

Phenylalanine standard solution (V_{Ph}). Weigh 100 mg of L-phenylalanine into a 100-ml measuring flask and fill to the mark with methanol–0.1 *M* hydrochloric acid (1:1).From this stock solution the standard solutions are prepared for the working range required. Dilute 200 µl, 400 µl, 600 µl, etc. of the stock solution to 10 ml with hydrochloric acid (10 g of acid per litre of solution)–methanol (1:1). Thus 0.1–0.3% solutions of the L-enantiomer relative to the 200 mg of D-phenylalanine are obtained.

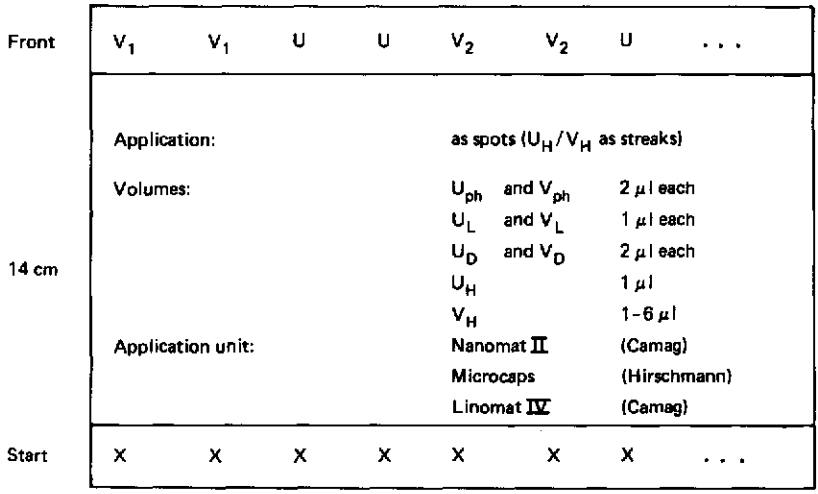


Fig. 10. The application pattern for direct quantification of the enantiomers of phenylalanine (Ph). *tert.*-leucine (L), 5,5-dimethylthiazolidine-4-carboxylic acid (D) and hydroxyphenylalanine (H).

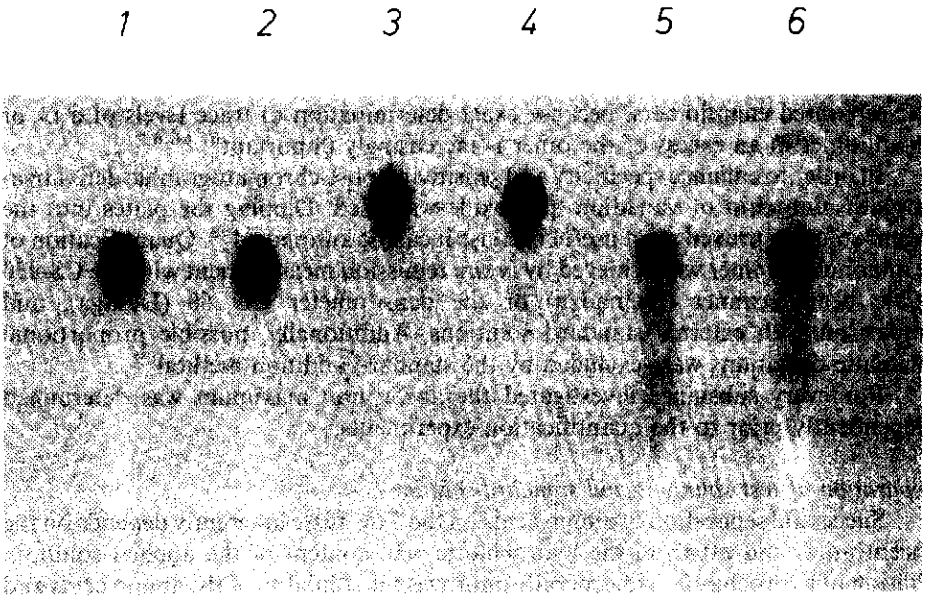


Fig. 11. Photograph of a thin-layer chromatogram on Chiralplate with eluent A. Spots: 1 = D-phenylalanine; 2 = 1% L-Phe in D-Phe; 3 = L-*tert.*-leucine; 4 = 1% D-*tert.*-leucine in L-*tert.*-leucine; 5 = D-5,5-dimethylthiazolidine-4-carboxylic acid; 6 = 3% L-5,5-dimethylthiazolidine-4-carboxylic acid in D-5,5-dimethylthiazolidine-4-carboxylic acid.

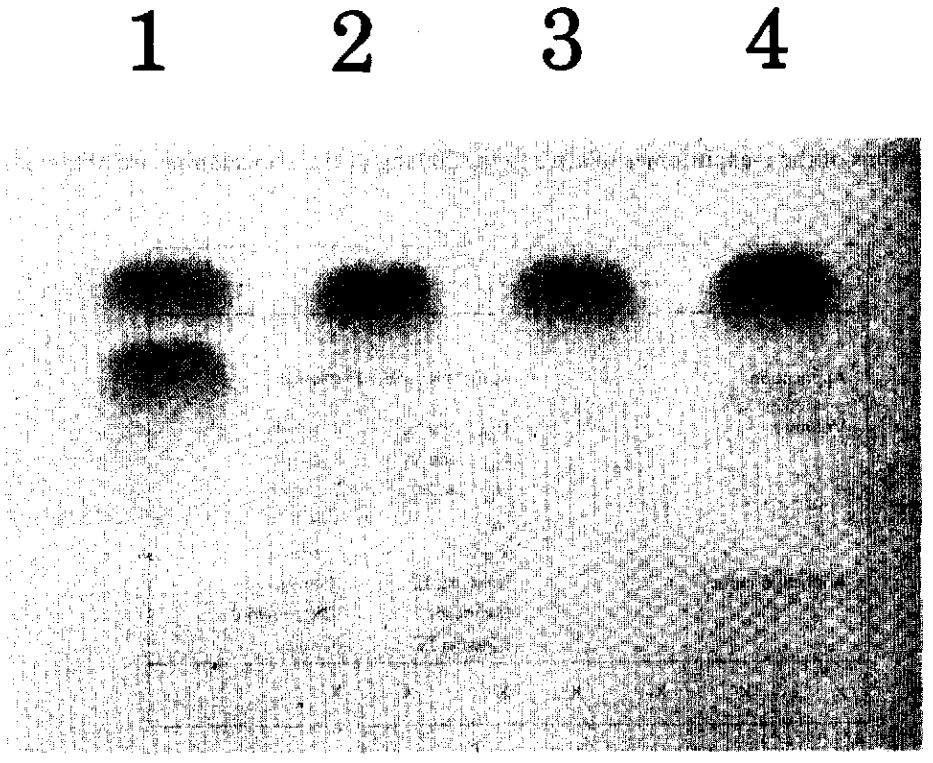


Fig. 12. Photograph of a thin-layer chromatogram on Chiralplate with eluent E. Spots: 1 = D,L-hydroxyphenylalanine; 2 = L-hydroxyphenylalanine; 3 = 3% D-hydroxyphenylalanine in L-hydroxyphenylalanine; 4 = 10% D-hydroxyphenylalanine in L-hydroxyphenylalanine.

tert.-Leucine test solution (U_L). Dissolve 200 mg of L-*tert.*-leucine in 10.0 ml of 50% methanol.

tert.-Leucine standard solution (V_L). Dissolve 100 mg of *tert.*-leucine in 100 ml of 50% methanol. Dilute 200 µl, 400 µl, 600 µl, etc. of this stock solution to 10 ml with 50% methanol to obtain 0.1–0.3% D-enantiomer relative to 200 mg of L-*tert.*-leucine.

5,5-Dimethylthiazolidine-4-carboxylic acid test solution (U_D). Add 500 µl of concentrated hydrochloric acid to 500 mg of D-5,5-dimethylthiazolidine-4-carboxylic acid and make up to 10 ml with isopropanol.

5,5-Dimethylthiazolidine-4-carboxylic acid standard solution (V_D). Add 500 µl of concentrated hydrochloric acid to 100 mg of L-5,5-dimethylthiazolidine-4-carboxylic acid and make up to 100 ml with isopropanol. Add 500 µl of concentrated hydrochloric acid to 500 µl, 1000 µl, 1500 µl, etc. of this stock solution, and make up to 10 ml with isopropanol. These solutions correspond to 0.1–0.3% of the L-enantiomer relative to 500 mg of D-5,5-dimethylthiazolidine-4-carboxylic acid.

Hydroxyphenylalanine test solution (U_H). Weigh 300 mg of L-hydroxyphenylalanine into a 10-ml measuring flask and fill to the mark with methanol.

Hydroxyphenylalanine standard solution (V_H). Dissolve 30 mg of D-hydroxyphenylalanine in 100 ml of methanol, and 1 µl, 2 µl, 3 µl, etc. of this stock solution correspond to 1–3%of the D-enantiomer relative to 300 mg of L-hydroxyphenylalanine.

Application pattern and sample volumes

For direct quantification of the enantiomers of phenylalanine, *tert.*-leucine, 5,5-dimethylthiazolidine-4-carboxylic acid and hydroxyphenylalanine the pattern of application shown in Fig. 10 has been used.

Chromatographic conditions

In general, the separation conditions for quantitative evaluation were similar to those for qualitative enantiomer separations by TLC. Any differences will be explained below. The plates were TLC precoated Chiralplates (Cat. No. 811 58, Macherey-Nagel); sizes, 20 cm × 20 cm; layer thickness, 0.25 mm. They were activated for 15 min at 110°C in a drying cabinet prior to use. The details of the eluents and detection procedures were as given above for the qualitative separation.

Spectrophotometric conditions

Instrument, double-beam TLC scanner CS 930 (Shimadzu, Japan); measuring set-up, monochromator —sample (remission); light source, tungsten lamp; wavelength, see under remission-location curves in figures; measuring area, 1.2 mm × 3 mm; feed, 0.05 mm.

Instrument, densitometer CD 60 (Desaga, F.R.G.); measuring set-up, monochromator —sample (remission); light source, tungsten lamp; wavelength, see under remission-location curves in figures; measuring area, 6.0 mm × 0.4 mm; feed, 0.1 mm.

For the evaluation, the absorption curve is measured in the chromatographic direction. The measured peak areas resp. peak heights, plotted against the amount of sample per spot, give the calibration lines.

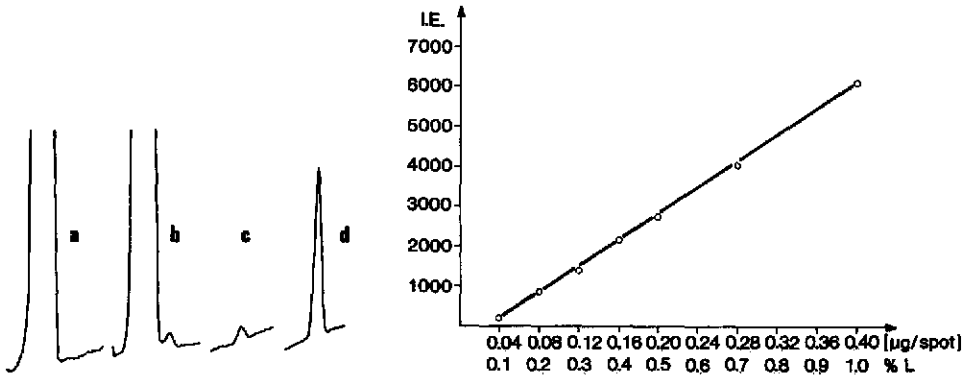


Fig. 13. Remission-location curves; (a) D-phenylalanine (Phe); (b) D-Phe spiked with 0.1% L-Phe; (c) 0.1% L-Phe; (d) 1% L-Phe. Conditions: eluent A; λ = 540 nm.

Fig. 14. Calibration line for L-phenylalanine (V). I.E. = integration units; $y = -463 + 16\,349\,x$; $r = 0.9992$; $S_{x_0} = 0.0038$ µg/spot (69); λ = 540 nm.

RESULTS

For better visualization of zones in Figs. 11 and 12, samples were spiked with more of the D-enantiomer than cited for the remission-location curves.

Phenylalanine

Fig. 13 shows, among others, the remission-location curve for two standard solutions with widely different L-phenylalanine concentrations. The calibration line in Fig. 14 shows that quantitative determinations of L-phenylalanine in D-phenylalanine is possible in a working range of 0.04–0.4 µg/spot without any problem.

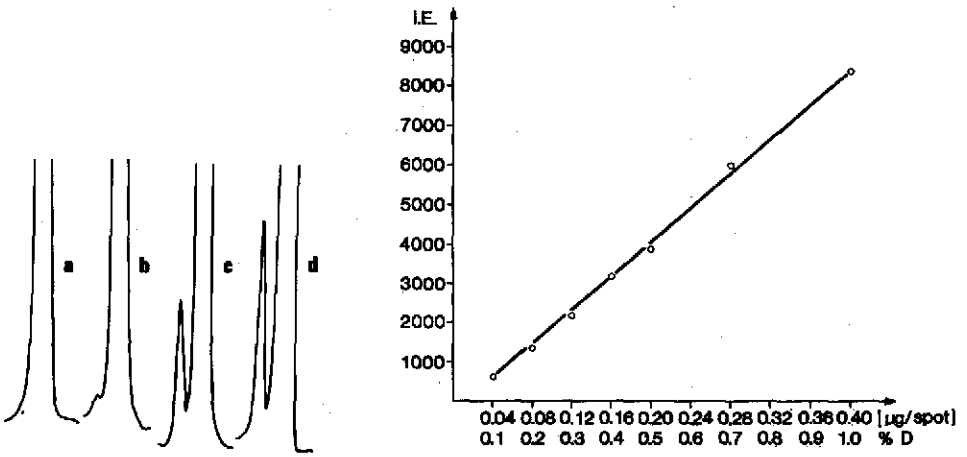


Fig. 15. Remission-location curves: (a) L-*tert.*-leucine (Degussa); (b) L-*tert.*-Leu + 0.1% D-*tert.*-Leu; (c) L-*tert.*-Leu + 1% D-*tert.*-Leu; (d) external reference sample. Conditions: eluent C; λ = 520 nm.

Fig. 16. Calibration line for D-*tert.*-leucine. I.E. = integration units; $y = -375 + 21\,984x$; $S_{x_0} = 0.0060$ µg/spot; $r = 0.9980$; λ = 520 nm.

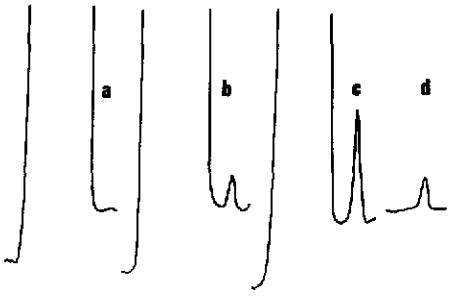


Fig. 17. Remission-location curves; (a) D-5,5-dimethylthiazolidine-4-carboxylic acid; (b) and (c) D-5,5-dimethylthiazolidine-4-carboxylic acid + 0.1% and 0.5%, respectively, of the L-enantiomer; (d) 0.1% L-5,5-dimethylthiazolidine-4-carboxylic acid. Conditions: eluent A; λ = 370 nm.

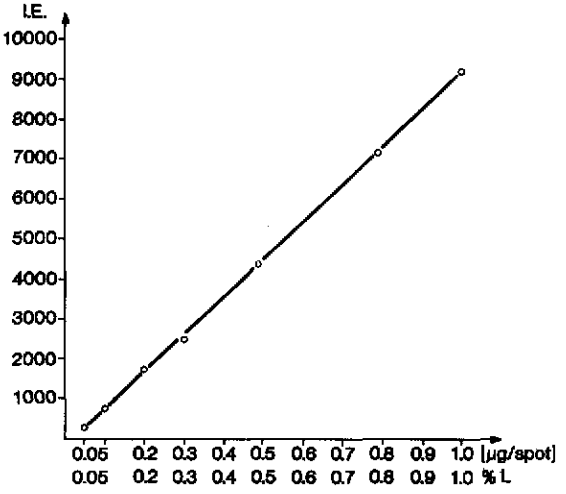


Fig. 18. Calibration line for L-5,5-dimethylthiazolidine-4-carboxylic acid. I.E. = integration units; $y = -192 + 9224x$; S_{x_0} = 0.015 µg/spot; r = 0.9985; λ = 370 nm.

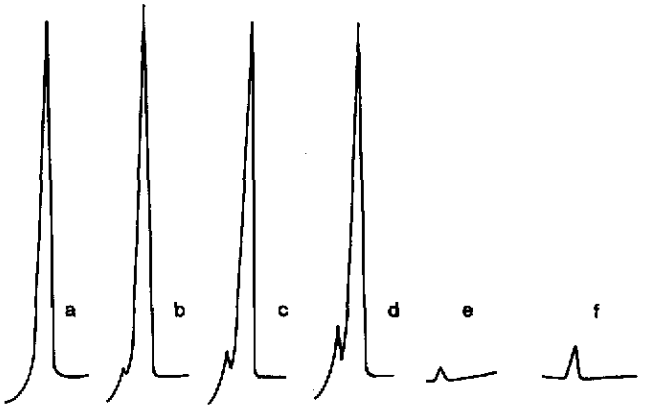


Fig. 19. Remission-location curves: (a) L-hydroxyphenylalanine; (b) 1% D-hydroxyphenylalanine in the L-enantiomer; (c) 3% D-hydroxyphenylalanine in the L-enantiomer; (d) 5% D-hydroxyphenylalanine in the L-enantiomer; (e) 1% D-hydroxyphenylalanine; (f) 3% D-hydroxyphenylalanine.

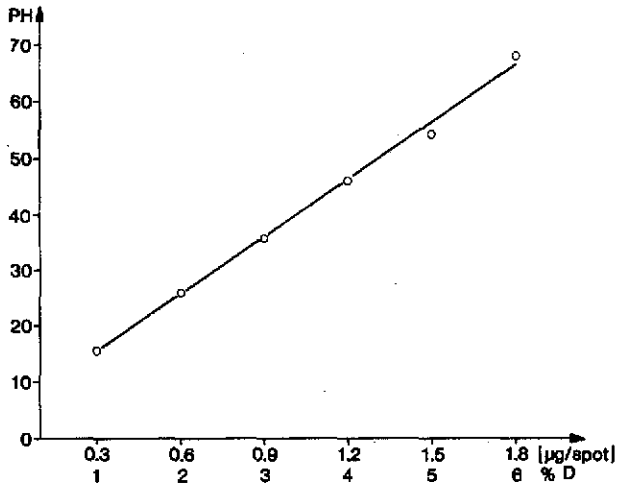


Fig. 20. Calibration line for D-hydroxyphenylalanine (V). PH = peak height; $y = 4.91 + 34.2x$; $r = 0.9981$; $S_{x_0} = 0.038$ µg/spot; $\lambda = 590$ nm.

tert.-Leucine

The remission-location curve (Fig. 15) and the calibration line (Fig.16) of *tert.*-leucine show clearly that the D-enantiomer can also be determined with high sensitivity in the L-amino acid.

5,5-Dimethylthiazolidine-4-carboxylic acid

The remission-location curve (Fig. 17) and the calibration line (Fig. 18) show that even 0.1–1% of the L-enantiomer can easily be quantitated.

α-Hydroxyphenylalanine

The remission-location curve (Fig. 19) and the calibration curve (Fig. 20) show good evaluation of the amount of D-enantiomer in the working range 1–6%.

CONCLUSION

The experimental results justify the use of a densitometer for quantitation of antipodes at trace levels. Contrary to the "classical" methods GC and HPLC, TLC is very well suited for simultaneous separation of several samples and can therefore be used for economical routine analyses. Further optimization of separation conditions may even improve the actual detection limits of $\geqslant 0.1\%$ of the minor enantiomer. Racemic separations of other classes of compounds —be they derivative or nonderivative— will be studied in our laboratory.

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