Journal of Pharmaceutical & Biomedical Analysis
Vol. 8, No. 3, pp. 259–269, 1990
Printed in Great Britain

0731–7085/90 $3.00 + 0.00
© 1990 Pergamon Press plc

Importance of enantiomeric purity and its control by thin-layer chromatography

J. MARTENS* and R. BHUSHAN

Fachbereich Chemie, Universität Oldenburg, Postfach 2503, D-2900 Oldenburg, FRG

Abstract: Methods for the direct resolution of enantiomers are important and are necessary for pharmaceutical and biomedical analysis, synthetic and mechanistic studies and various other fields. The present paper deals with the results of recent approaches, such as ligand exchange, ion exchange and steric interactions, providing direct resolution of enantiomers of a variety of compounds by thin-layer chromatography. General aspects of various methods for analysis of enantiomeric purity and resolution have been compared.

Keywords: *Thin-layer chromatography; enantiomers; dansyl-, phenylthiohydantoin-,α-methyl-,* N*-alkyl- and* N*-formyl-amino acids; dipeptides; α-hydroxy carboxylic acids; purine nucleosides.*

Introduction

Macroscopic life shows a high degree of symmetry but at the molecular level asymmetry dominates. The interaction of biological molecules with a remarkable stereospecificity of one biomolecule for another has always been fascinating. Stereoselective discrimination here appears in extreme form. Chiral discrimination is also known for drugs and substrates and is based upon complementary chemistry. By the key lock principle this can be visualized, not as something static but as a dynamic process of mutual adaptation, an "embracement" between drug/substrate on one side and enzyme or receptor on the other [1]. The leading scientist in the field is Ariens who made clear that "stereoselectivity of biological systems and stereospecificity in the action of bioactive chiral xenobiotics is a natural matter" [1]. The idea of stereochemistry and stereoselectivity in biological processes goes back to Pasteur, van't Hoff and Le Bell, more than 100 years ago. Pasteur stated in his memoirs [2]: "Most organic products, the essential products of life, are asymmetric and poses such asymmetry that they are not superimposable on their images . . . This establishes perhaps the only well marked line of demarkation that can at present be drawn between the chemistry of dead matter and the chemistry of living matter".

Chirality, stereoselectivity and the stereospecific formation of chemicals are characteristics of nature. Most of the xenobiotics obtained by classical organic synthesis are chiral also. However, in contrast to natural products, synthetics as prepared by chemists are usually obtained as mixtures of stereoisomers such as racemates or diastereomers. This situation is changing [1] with the development of asymmetric synthesis in many laboratories, both academic [3–8] and industrial [9]. Asymmetric syntheses and the separation of stereoisomers are laborious and expensive and have traditionally been considered as some of the more difficult problems in inorganic chemistry and separation science. As a consequence, a significant portion (approximately 25%) of the most widely prescribed drugs listed in the handbook of Kleemann and Engel [10] are still sold as mixtures of stereoisomers (Fig. 1). It is perhaps not surprising that early data on the different physiological behaviour are found in the taste and smell of enantiomers [13–19] (selected examples are listed in Table 1).

The enantioselective action of chiral drugs has been reviewed by several authors [1, 20–28]. The first reported drug exhibiting stereoselective binding to human serum albumin (HSA) was oxazepam succinate. Thus in 1975 Müller and Wollert [29] found the *S*-enantiomer has about 35 times higher binding affinity than the *R*-isomer. Since then the

* Author to whom correspondence should be addressed.

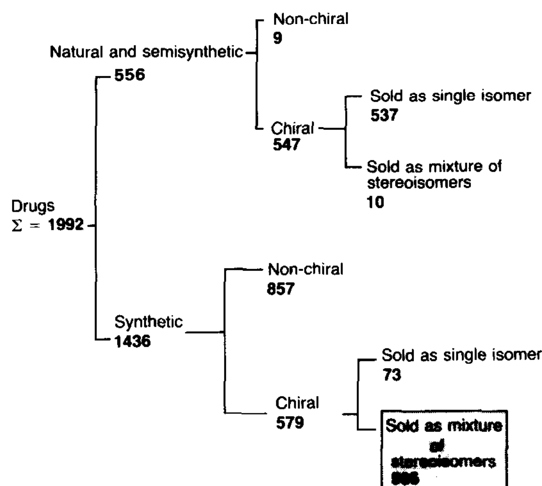

Figure 1
Chirality of drugs listed in ref. 10 and their application as sterically uniform compounds or isomeric mixtures. Figures in bold represent the number of drugs.

Table 1
Enantioselectivity of the taste of amino acids

Amino acid	Taste/enantiomer D	L
Asparagine	Sweet	Tasteless
Histidine	Sweet	Tasteless
Isoleucine	Sweet	Bitter
Leucine	Sweet	Bitter
Tryptophan	Sweet	Tasteless
6-Chlorotryptophan	Sweet*	Tasteless
Tyrosine	Sweet	Bitter

*The D-(+)-enantiomer has been reported to be one thousand times sweeter than sucrose [11, 12].

investigation of several other 3-substituted 1,4-benzodiazepines confirmed [30–32] that *S*-enantiomers are preferentially bound to HSA.

Very often, scientists dealing with mixtures of isomers are not aware that they are composed of an active and an "inactive" isomer and they feel that the latter may be ignored, but there is abundant evidence for chirodiastaltic interactions [33]. In two crystals, one exclusively from *S* (or *R*) and the other from *S and R* species the molecules adopt different modes of packing. They interact differently and have different lattice energies which are reflected by different melting points of the two crystals.

Stereospecificity occurs also when enzymes or peptides are involved. Enantiomers do not differ only in the metabolic rates in which they are processed in a biological system, they may even be metabolized along different pathways [23], or may bind to different receptor types leading to different biological responses. Ariens [1] stated earlier: "It is surprising that many biologists, pharmacokineticists and clinical pharmacologists operate on a pre-Pasteur level with regard to stereochemistry. This implies that, unless having been explicitly noticed, they tend to deal with mixtures of isomeres as if one compound were involved. Once the mixture is given the INN name by the WHO that name and the brand name stand for 'the drug', 'the pharmacon', 'the active compound'. It is not surprising that a physician, presented with such a drug under a brand name or the INN name is unaware that he has received a mixture of stereoisomers. It is understandable but unacceptable that he then makes mistakes."

Most modern handbooks on pharmacology, pharmacokinetics or toxicology do not even mention the terms related to stereoselectivity!

Racemates may be regarded as drugs containing 50% impurity [34]. FDA and other national drug agencies involved in the registration of new active ingredients expect pharmacologists nowadays to present full information on the stereochemistry and stereoselectivity of biologically active compounds, including the necessary stereoselective analytical methods. This review deals with a new and simple direct analytical method for the separation of various enantiomers. Special emphasis is given to the analysis of compounds which are of interest to pharmacologists and medicinal chemists.

Methods for Analysis of Enantiomeric Purity

Enantiomeric purity, i.e. the purity with respect to the content of only one enantiomer, is an entirely different concept in comparison with the purity of organic compounds as determined commonly. The methods used for establishing enantiomeric purity/composition may or may not provide separation of the two antipodes. Polarimetry, NMR, isotopic dilution, differential scanning calorimetry (DSC), and enzymatic reactions do not provide separation of enantiomers but are used for determination of enantiomeric composition. All these methods except NMR require data from the optically pure enantiomer for comparison.

Polarimetry, though most widely used, requires knowledge of the specific rotation of the

compound in optically pure form. The specific rotation is highly dependent on solvent, concentration and temperature. Since the optical purity is based on experimentally determined chiroptical properties it may be associated with systematic errors and may not always correspond to the actual enantiomeric composition or enantiomeric purity. Optical purity is linearly related to enantiomeric purity only when there is no molecular association between the enantiomers in solution [35].

Determination of enantiomeric purity by NMR requires the conversion of enantiomers into diastereomers by reaction with a suitable chiral reagent. Alternatively, a chiral solvent such as 2,2,2-trifluoro-1-phenyl ethanol is used which induces a chemical shift difference between the enantiomers (of otherwise identical nuclei) and accordingly the enantiomer ratio can be obtained by integration. The solvent induced chemical shift difference is a consequence of the preferential interaction of one of the enantiomers with the chiral solvent. However, the enantioselective interaction with the chiral solvent is often too small to be of practical use. In the isotopic dilution method, the sample of unknown enantiomeric purity is mixed with an isotopically labelled racemate of the same compound. The mixture is subjected to recrystallization to yield a new sample with another optical purity and specific rotation; by knowing the isotopic content of this sample, the optical purity of the original sample can be calculated. Thus the method depends upon the determination of specific rotation (polarimetry) and isotope content.

Differential scanning calorimetry is based on the determination of the energy absorbed or evolved by a sample as a function of temperature. The method is only applicable to solid compounds.

Certain enzymatic reactions are highly stereoselective. Oxidation and decarboxylation constitute the basis of enzymatic catalysis. Though enzyme catalysis of amino acid transformations is particularly useful for exact determination of high enantiomeric purity, it results in the loss of one of the enantiomers.

To achieve separation of enantiomers, fractional crystallization [36] and seeding methods [37] have been used. None of these has general applicability and all are relatively time consuming and tedious. They normally require optically pure agents for derivatization and they often fail to afford total separation of enantiomers.

Chromatography provides practically useful methods for the determination of enantiomeric composition or purity and the resolution of enantiomers on a very small scale with relative ease and efficiency. Gas chromatography (GC) and high-performance liquid chromatograpy (HPLC) have particularly been employed in recent years for the resolution of enantiomers of a variety of compounds [38–40]. In GC, the separation is generally achieved either by using the chiral stationary phase (CSPs) or by derivatizing a racemate with a chiral molecule into a pair of diastereomers, which are then resolved [41–45]. The use of the chiral derivatization method poses several limitations such as: (a) active functional groups or chiral reagents for forming diastereomers are required and it is sometimes very difficult to obtain optically pure chiral reagents: (b) individual enantiomers have different reaction rates; and (c) the diastereomeric mixture must be chemically and stereochemically stable. GC is generally best suited to compounds containing polar functional groups like amides, esters and alcohols, and such compounds often require a high column temperature. The column temperature may cause racemization of the solute during analysis, and another dilemma is that the separation factor obtained increases with decreasing temperature [46]. HPLC makes use of either CSPs or chiral additives in mobile phase in conjunction with achiral or chiral stationary phases. In addition, derivatizing a pair of enantiomers with chiral agents and using the achiral or chiral stationary phase constitutes another approach for separation of enantiomers. As already mentioned, performing *indirect* enantioseparation always requires determination of the actual purity of the chiral reagent, for example, by reaction with optically pure selectand or with a selectand of known enantiomeric composition. This may lead to further risk of erratic analytical results.

At present chiral compounds and bonded phases are by no means cheap, since the development costs are formidable. The CSPs which are already on the market [47] are relatively new and their performance is yet to be fully assessed.

Both GC and HPLC methods are sensitive but time consuming, costly, restricted to certain classes of compounds, and extensive purification is needed before analysis of samples

from syntheses or solutions of pharmaceutical preparations such as capsules or tablets. Nevertheless, thin-layer chromatography (TLC) can be considered to have several advantages over other methods of enantiomeric resolution and control of purity [48–50].

Advantages of TLC

TLC provides a simple, sensitive, inexpensive and direct method for resolution and analytical control of enantiomeric purity. It allows densitometric scanning for quantitation and the components of the mixture can be easily eluted and used subsequently. The inert character of thin layer material is very advantageous. The polarity of the solvent or the type of solvent mixture can be changed in a matter of minutes. Development time is shown, and with easy change of the mobile phase, TLC is probably the easiest chromatographic method to set up for a specific compound.

Due to accurate and precise spotting techniques, instrumentalized development devices and sophisticated densitometers, the detection and quantification of analytes on TLC plates have progressed considerably in recent years. The linking of TLC detectors to computing integrators or IBM type PCs provides quantitative data from the TLC plates and is now comparable to obtaining such information from column chromatography. Finally, it may also be mentioned that studies are starting to appear on the use of mass spectrometry (MS) detection in TLC; both SIMS (Secondary Ion Mass Spectrometry) and laser MS have successfully been employed as ionization modes [51].

Probably one of the most advantageous features of TLC, as opposed to other chromatographic methods, is that a number of samples can be handled simultaneously [52]. It is evident that simultaneous procedure, in principle, yields a higher precision than sequential analysis.

Analytical Applications

Proteinogenic and non-proteinogenic amino acids

The direct TLC resolution of enantiomers of certain amino acids was achieved as early as 1965 by Contractor and Wragg [53] using cellulose plates. The lowest concentration of DL-5-hydroxy tryptophan detected was 0.05–0.1 μg. The hR_f values for the amino acids resolved and chromatographic conditions are shown in Table 2. Recently, Yuasa *et al.* [54] have proposed that under hydrophobic conditions, the hydrophobic moities of D-glucose units in cellulose are axial, and thus cellulose gives rugged and helical conformation so that all amino acids migrate more slowly because of their tight interaction with the D-glucose units. Consequently, the enantiomers are resolved because of their interaction difference. Yuasa *et al.* [54, 55] have been able to resolve racemic tryptophan, isoleucine, valine, norvaline, norleucine and alanine using microcrystalline cellulose plates (from Funakoshi Yakuhin Co., Japan) and pyridine– ethanol–water (5:1:1) as mobile phase.

Table 2
Resolution of DL-amino acids on cellulose plates [53]

Amino acid	hR_f value D	hR_f value L
DL-Tryptophan	62	52
DL-5-Hydroxy tryptophan	54	47
DL-6-Hydroxy tryptophan	41	36

Plates: 20 × 20 cm × 0.3 or 0.4 mm cellulose (Whatman).
Development: butanol–pyridine–water (1:1:1, v/v/v), 1 h.
Detection: Ehrlich's reagent.

Based on the work of Davankov *et al.* [56, 57] who modified commercial HPLC columns by grafting alkyl derivatives of α-amino acids such as *n*-decyl-L-histidine or *n*-hexa decyl-L-proline, onto the resin for enantiomeric resolution, a new chiral selector (2*S*, 4*R*, 2′*RS*)-*N*-(2′-hydroxy dodecyl)-4-hydroxy proline [58] was used (Fig. 2) for the resolution of enantiomeric amino acids at Degussa AG [59–62]. The ligand exchange TLC method of Günther and Martens [59–62] takes advantage of stereoselectivity of the Cu^{2+} complex of this

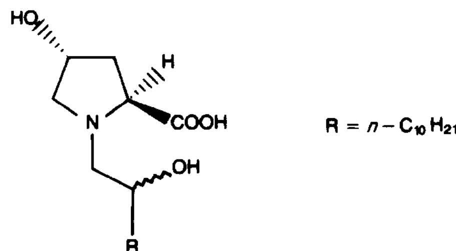

Figure 2
Structure of the chiral selector used by Günther and Martens [58, 62].

Table 3
Ligand exchange TLC resolution of enantiomeric proteinogenic and non-proteinogenic amino acids [59–68]

Amino acid	hR_f value (configuration)		Eluent
Isoleucine	37 (2*R*, 3*R*)	44 (2*S*, 3*S*)	A
Phenylalanine	38 (D)	45 (L)	A
Tyrosine	34 (D)	26 (L)	B
Tryptophan	39 (D)	45 (L)	A
Proline	40 (D)	59 (L)	B
Glutamine	53 (D)	37 (L)	A
Alanine	69 (D)	73 (L)	D
Aspartic acid	50 (D)	55 (L)	A
Glutamic acid	54 (D)	59 (L)	A
Leucine	53 (D)	63 (L)	C
Methionine	54 (D)	59 (L)	A
Valine	54 (D)	62 (L)	A
Serine	73 (D)	76 (L)	D
t-Leucine	40 (D)	51 (L)	A
Norleucine	53 (D)	62 (L)	A
allo-Isoleucine	51 (D)	61 (L)	A
Norvaline	49 (D)	56 (L)	A
2-Phenylglycine	57 (D)	67 (L)	A
Ethionine	52 (D)	59 (L)	A
(1-Naphthyl)-alanine	49 (D)	56 (L)	A
(2-Naphthyl)-alanine	44 (D)	59 (L)	A
O-benzylserine	54 (D)	65 (L)	A
O-benzyltyrosine	48 (D)	64 (L)	A
Methioninesulphone	62 (D)	66 (L)	A
Selenomethionine	53 (D)	61 (L)	A
Dopa	58 (D)	47 (L)	B

Development: A, methanol–water–acetonitrile (1:1:4, v/v/v), 30 min; B, methanol–water–acetonitrile (5:5:3, v/v/v), 60 min; C, methanol–water (1:8, v/v); D, acetone–methanol–water (10:2:2, v/v/v).
Detection: 0.3% ninhydrin in acetone.

hydroxy proline derivative, and has a relatively high degree of selectivity.

The method was capable of resolving enantiomers in trace amounts, for example, the lowest level of detection of D-dopa in L-dopa samples was >0.25% [62], and that for D-penicillamine in a DL-mixture was >0.5% [63]. Resolution of several proteinogenic and non-proteinogenic amino acids has been achieved without derivatization using this ligand exchange TLC; Table 3 shows some typical results and conditions. However, with this technique more than 100 racemate separations have been accomplished by Günther and co-workers [59–68]. The ligand exchange TLC plates have now been made commercially available through Macherey-Nagel and E. Merck under the trade names of Chiralplate® and Chir®. Brinkman and Kamminga [69] further simplified the experimental conditions and resolved eight DL-amino acids on Chiralplate® of size 4 × 6 cm, in 4 min, in water–acetonitrile (1:4, v/v). Chiralplate® has also been used in deciding the enantiomeric purity of synthetic racemic homomethionine (2-amino-5-thiomethyl pentanoic acid) which was resolved enzymatically or chemically [70].

Using the ion exchange approach, Bhushan *et al.* [71] resolved enantiomeric amino acids on thin silica gel plates impregnated with brucine (Table 4).

Table 4
Resolution of enantiomeric amino acids on brucine impregnated silica gel plates [71]

Amino acid	hR_f values: Pure L	Resolved D	Resolved L
Alanine	53	18	53
Isoleucine	35	16	35
Methionine	29	18	29
Phenylalanine	40	27	40
Serine	50	12	50
Threonine	29	16	29
Tryptophan	31	17	31
Tyrosine	29	22	29

Development: *n*-butanol–acetic acid–chloroform (3:1:4, v/v/v), 30 min.
Detection: 0.2% ninhydrin in acetone.

α-Methyl-amino acids

Table 5 shows some typical results for the resolution of enantiomers of α-methyl-amino acids on Chiralplate® [67] without any derivatization step. Brückner *et al.* [72] investigated the influence of change of composition of mobile phase on the resolution factors of the enantiomers of α-methyl-α-amino acids having an aromatic side chain, while Neuzil *et al.* [73] investigated the rôle of structural factors which favoured the TLC separation with a large series of racemic amino acids and their derivatives, using the TLC techniques of Günther and Martens.

Table 6
Resolution of enantiomers of *N*-alkyl- and *N*-formyl-amino acids [64, 65]

Racemic derivative	hR_f values D	L	Eluent
N-methylleucine	57	49	A
N-methylphenylalanine	50	61	A
N-methyl-*m*-tyrosine	36	52	B
N-methylvaline	70	65	B
N,*N*-dimethylphenylalanine	55	61	B
N-formyl-t-leucine	48 (+)	61 (−)	A

A, methanol–water–acetonitrile (1:1:4, v/v/v); B, methanol–water–acetonitrile (5:5:3, v/v/v).

N-alkyl- and N-formyl-amino acids

Resolution of enantiomers of *N*-alkyl- and *N*-formyl-derivatives of various amino acids on Chiralplate® [64] is given in Table 6. Gont and Neundorf [74] resolved enantiomeric *N*-carbamyl tryptophan under identical conditions. It is reported [74] that *N*-substitution reduces the sensitivity of the ninhydrin reaction, therefore other appropriate visualization techniques must be applied to every particular case. The detection of *N*,*N*-dimethylphenylalanine has been achieved with iodine [64].

Table 5
Resolution of enantiomeric α-methyl-amino acids [67]

Racemic mixture	hR_f		Eluent
α-Methylmethionine	56 (D)	64 (L)	A
α-Methylserine	67 (D)	56 (L)	B
α-Methyltyrosine	63 (D)	70 (L)	A
α-Methylphenylalanine	66 (D)	53 (L)	A
α-Methyldopa	66 (D)	46 (L)	B

A, methanol–water–acetonitrile (1:1:4, v/v/v); B, methanol–water–acetonitrile (5:5:3, v/v/v).

α-Hydroxy carboxylic acids

Jork and Kany [75] for the first time resolved enantiomers of 3,4-dihydroxy-mandelic acid and vanilic acid during the analysis of enantioselective degradation of the biogenic catecholamines, norepinephrine (noradrenaline) and epinephrine (adrenaline) using dichloromethane–methanol (45:5, v/v) and post-chromatographic detection with 2,6-dichloroquinone-4-chlorimide. Günther [48] has reported the resolution of a number of α-hydroxy carboxylic acids on Chiralplate®; Table 7 shows the R_f values for some typical resolutions. Vanadium pentoxide has especially been found useful for post-chromatographic derivatization [76].

Table 7
Resolution of α-hydroxy acids [48]

	hR_f values			
	Chiralplate*		Chir†	
Racemic compound	D	L	D	L
Mandelic acid	46	53	55	60
3-Hydroxy-mandelic acid	34	39	67	72
4-Hydroxy-mandelic acid	32	36	69	74
3,4-Dihydroxy-mandelic acid	32	38		
4-Bromo mandelic acid			38	44
4-Chloro mandelic acid			40	47
Vanillylmandelic acid	49	55		
Hydroxyisoleucine	56	63		
Hydroxyphenylalanine	56	62		
Hydroxyvaline	52	60		
4-Hydroxy-3-methoxy-mandelic acid			50	71
Methoxyphenylacetic acid			53	58

*Dichloromethane–methanol (9:1), migration distance 13 cm.

†0.05 M Potassium dihydrogen phosphate in methanol–water–acetonitrile (5:5:2, v/v/v), migration distance 7 cm; detection, manganese chloride–sulphuric acid/30 min 120°C.

Dansyl- and PTH-amino acids

Dansyl- and phenylthiohydantoin amino acids (Fig. 3) are used for the identification of amino acid sequences of proteins by manual and automated procedures.

The early finding of direct optical resolution of amino acids by cellulose TLC [53], led scientists to use other natural chiral polymeric materials for TLC resolution of enantiomers. Cyclodextrins, formed by the microbial (*Bacillus maccrans*) degradation [77] of starch, were used for chiral resolutions as mobile phase additives in TLC experiments [78–80]. Alak and Armstrong [81] used β-cyclodextrin bonded silica gel plates to resolve enantiomers of dansyl-amino acids, the results are shown in Table 8. Cyclodextrin is believed to form some sort of inclusion complexes with the two enantiomers based on hydrophobic interaction and its chiral structure leading to direct separation. Resolution of enantiomeric dansyl-amino acids has also been achieved by ligand exchange TLC using a copper(II) complex of *N*,*N*-di-*n*-propyl-L-alanine in one-[82] and two-dimensional modes [83]. In the first dimension dansyl-amino acids were separated in a non-chiral mode by using a convex gradient elution with sodium acetate buffers and varying concentrations of acetonitrile. In the second dimension, the plates were treated with *aq*-acetonitrile–sodium acetate buffers. Marchelli *et al.* [84] used copper(II) complexes of the ligand prepared from L-amino acid (alanine, valine or phenylalanine) and 1,2-ethane- or 1,3-propane diamine for one- and two-dimensional TLC resolution of dansyl-amino acids. The chiral selector was used as an impregnating agent or as an additive in the eluent (Table 9). Recently, Sinibaldi *et al.* [85] resolved DL-dansyl-amino acids on reversed-phase TLC plates pre-treated with a copper(II) complex of poly-L-phenyl alaninamide (Fig. 4) under isocratic conditions; the hR_f values are represented in Table 10.

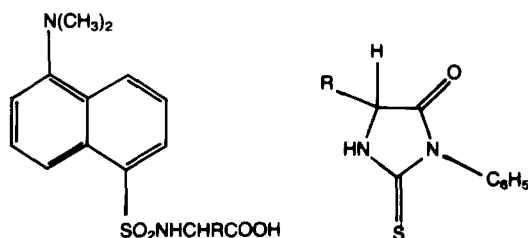

Figure 3
Structures of dansyl- and PTH-amino acids.

Table 8
Resolution of enantiomeric dansyl-amino acids on β-cyclodextrin plates [81]

Dansyl-amino acid	hR_f value D	hR_f value L	Mobile phase
Leucine	49	66	2:3
Methionine	28	43	1:3
Alanine	25	33	1:3
Valine	31	42	1:3

Mobile phase: methanol–1% triethyl ammonium acetate (pH 4.1); detection by fluorescence.

The enantiomers of phenyl thio hydantoin amino acids have been resolved by Bhushan and Ali [86] using silica gel plates impregnated with (+)-tartaric acid or ascorbic acid; the results are shown in Table 11.

Dipeptides

The direct resolution of enantiomeric and diastereomeric dipeptides (i.e. without deriv-

Table 9
Resolution of enantiomeric dansyl-amino acids using a copper complex of phenylalanine–1,2-ethanediamine [84]

Dansyl-amino acid	hR_f value D	hR_f value L	Eluent % Acetonitrile	pH
Glutamic acid*	40	24	33	6.8
Aspartic acid*	21	09	33	6.8
Serine*	51	41	50	7.5
Threonine*	52	47	50	7.5
Methionine*	40	34	50	7.5
Leucine†	38	47	50	7.5
Phenylalanine†	41	35	50	7.5

*With the complex (2 mM) in the eluent.
†Without the complex in the eluent.
Detection with fluorescent lamp (365 nm).

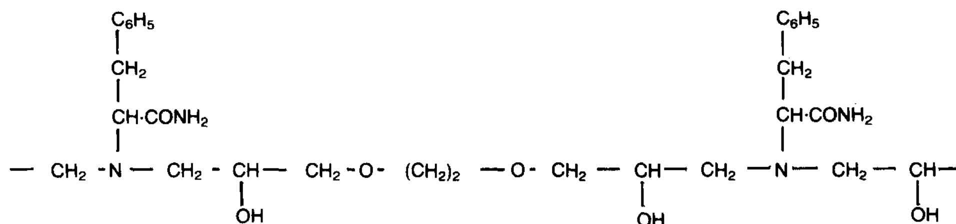

Figure 4
Poly-L-phenylalaninamide [88].

Table 10
Resolution of dansyl-amino acids on RP-18 plates impregnated with copper complex of poly-L-phenylalaninamide [85]

Dansyl-amino acid	hR_f value D	L	Eluent
Aspartic acid	40	35	A
Glutamic acid	63	55	A
Valine	37	33	B
Norvaline	41	36	B
Leucine	24	19	B
Methionine	25	18	B
Threonine	46	43	B
Phenylalanine	20	17	B

A, water–acetonitrile (7:3, v/v); B, water–acetonitrile (55:45); detection by UV (360 nm).

Table 11
Resolution of enantiomers of PTH-amino acids on tartaric acid impregnated plates [86]

PTH-amino acid	hR_f values D	L
Methionine	16	83
Phenylalanine	15	85
Valine	21	80
Isoleucine	15	92
Tyrosine	16	95
Threonine	30	85
Alanine	12	55
Serine	10	84

Solvent system: chloroform–ethyl acetate–water (28:1:1, v/v/v).
Detection: iodine vapours.

atization at any stage) has been successfully carried out for the first time by Günther *et al.* [71] on Chiralplate®. It was interesting to note that the antipodes with C-terminal L-configuration gave a lower R_f value than the corresponding enantiomer with C-terminal D-configuration (Table 12). Enantiomers of leu–leu were also resolved in Chir® using methanol–water–1-propanol (5:4:1, v/v/v) instead of methanol–water–acetonitrile used with Chiralplate®. Wang *et al.* [87] compared the separation and migration characteristics of dipeptides on Chiralplate® with those on cellulose, and used these TLC methods in identifying the distribution of stereomers during enantiomeric synthesis of dipeptides.

Table 12
Resolution of enantiomers of dipeptides on Chiralplate® [66]

Dipeptide	hR_f value Eluent A	Eluent B
Gly-D,L-Phe		63 (D) 57 (L)
Gly-D,L-Leu		60 (D) 53 (L)
Gly-D,L-Ile		61 (D) 54 (L)
Gly-D,L-Val		62 (D) 58 (L)
Gly-D,L-Try		55 (D) 48 (L)
D-Leu-L-Leu	19	48
L-Leu-D-Leu	26	57
D-Ala-L-Phe	21	59
L-Ala-D-Phe	26	65
D-Met-L-Met	29	64
L-Met-D-Met	33	71

A, methanol–water–acetonitrile (1:1:4, v/v/v); B, methanol–water–acetonitrile (5:5:3, v/v/v).

Heterocyclic compounds

Resolution of enantiomers of thiazolidine-4-carboxylic acid and 5,5-dimethyl thiazolidine-4-carboxylic acid formed by the condensation of formaldehyde with cysteine and penicillamine [63], respectively has been obtained on Chiralplate®. It is interesting that the reaction mixture can be applied directly onto the TLC plates. hR_f values for some of such heterocyclic compounds [48, 63] are recorded in Table 13.

Basic drugs

In an indirect approach, fluonoxaprofen chloride (FLOP-Cl), which is a non-steroidal anti-inflammatory agent, was used as a chiral derivatization reagent for the TLC resolution of enantiomers of certain drugs such as α-methylbenzylamine (α-MBA) and tranylcypromine (TCP) in biological fluids like plasma and urine [88]; resolution was achieved

Table 13
Enantiomeric resolution of some heterocyclic compounds [48, 63]

Racemic compound	hR_f value		Eluent
Thiazolidine-4-carboxylic acid	59 (D)	69 (L)	A
5,5-Dimethylthiazolidine-4-carboxylic acid hydrochloride	48 (D)	62 (L)	A
3-Amino-3,5,5-trimethylbutyrolactone-hydrochloride	50 (D)	59 (L)	A
Pipecolic acid	51 (D)	58 (L)	B

A, methanol–water–acetonitrile (1:1:4, v/v/v); B, acetone–methanol–water (10:2:2, v/v/v).

Table 14
Quantitative determination of selected enantiomers

Enantiomer detected	Lowest detection limit for the minor component (%)	Ref.
L-Phenylalanine in D-phe	0.1–1.0	94
D-t Leucine in L-isomer	0.1–1.0	94
L-5,5-Dimethyl thiazolidine-4-carboxylic acid	0.1–1.0	94
D-α-Hydroxy phenylalanine	1.0–6.0	48
L-Tryptophan in DL-try	0.01–0.1	70

on TLC plates precoated with silica gel with toluene–tetrahydrofuran–dichloromethane (5:1:1, v/v/v) as mobile phase. The method was efficient and sensitive, and the detection and assay limit for TCP was 1–2 ng ml^{-1} of the sample. In an attempt to investigate the effect of ion pair reagents such as heptane sulphonic acid (HSA) and sodium dodecyl sulphate (SDS) on TLC behaviour of drugs, Ruane and Wilson [89] performed ion pair RP-TLC on C-18 bonded and paraffin-coated silica plates for the following four basic drugs: practolol, i.e. (2*RS*)-3-(4-acetamidophenoxy)-1-isopropyl-amino-2-propanol; propanolol, i.e. (2*RS*)-1-isopropylamino-3-(1-napthyloxy)-2-propanol; (2*RS*,3*RS*)-1-(7-methylindan-4-yloxy)-3-iso-propylaminobutan-2-ol and *N*-2[(2RS)-3-*O*-cyanophenoxy-2-hydroxypropyl]aminoethyl-4-hydroxyphenyl-acetamide. However the studies did not involve any enantiomeric resolution.

Purine nucleosides

The resolution of α- and β-adenosine, β-deoxyadenosine and α- and β-thymidine has been studied [90] on Chiralplate®. The chromatograms were developed in methanol–water–acetonitrile (5:5:3, v/v/v) and detected under UV; the time required varied from 105 min for 23% acetonitrile to 35 min for 70–80% acetonitrile. α- and β-adenosine, and α- and β-deoxyadenosine separated successfully in all the solvents, while α- and β-thymidine ran with the solvent front. The β-nucleosides showed higher R_f values than the α-nucleosides. The method offers a simple and rapid resolution of anomers of adenine nucleosides.

Quantitative evaluation

It has also been demonstrated that quantitative determination of TLC separated antipodes can be a useful daily routine method in any laboratory. The quantitative determination for some of the enantiomers resolved on Chiralplate® [48, 91] and Chir® are represented in Table 14.

Conclusions

The resolution and assay of enantiomers of a variety of compounds requires highly efficient and sensitive methods in various fields. TLC provides one such simple method which can be useful for daily routine in any laboratory. It is believed that the innovation of new plate types and the continued development of theory, practice and instrumentation will lead eventually to TLC systems that are unrivalled for speed, versatility, accuracy, precision and sensitivity.

Acknowledgements — Thanks are due to Alexander von Humboldt Foundation (FRG) for awarding a research fellowship, and to the University of Roorkee (Roorkee, India) for granting leave of absence (to R.B.). Financial assistance from Fond der Chemischen Industrie and Degussa AG, Frankfurt a.M., is also gratefully acknowledged.

References

[1] E. J. Ariens, E. W. Wuis and E. J. Veringa, *Biochem. Pharmac.* **37**, 9–18 (1988).

[2] L. Pasteur, in *Memoirs by Pasteur, van't Hoff, Le Bel and Wislicensus* (G. M. Richardson, Ed.), pp. 1–33. American Book Company, New York (1901).
[3] D. Enders and W. Hoffmann, *Chem. unserer Zeit* **19**, 177–190 (1985).
[4] J. D. Morrison (Ed.) *Asymmetric Synthesis*, Vol. 1-5. Academic Press, Orlando (1983–1985).
[5] D. Seebach, G. Adam, T. Gees, M. Schies and W. Weigand, *Chem. Ber.* **121**, 507–517 (1988).
[6] M. Aglietto, E. Chiellini, S. D'Antone, G. Ruggeri and R. Solaro, *Pure Appl. Chem.* **60**, 415–430 (1988).
[7] W. Oppolzer, *Tetrahedron* **43**, 1969–2004 (1987).
[8] G. H. Posner, *Acc. Chem. Res.* **20**, 72–78 (1987).
[9] J. Martens, *Topics Curr. Chem.* **125**, 165–246 (1984).
[10] A. Kleemann and J. Engel, *Pharmazeutische Wirkstoffe*, Thieme, Stuttgart (1982); and *Pharmazeutische Wirkstoffe, Ergänzungsband 1982–1987*, Thieme, Stuttgart (1987).
[11] G. Chedd, *New Scientist* **62**, 299 (1974).
[12] E. C. Kornfeld, J. M. Sheneman and T. Suarez, *Chem. Abstr.* **72**, 30438c (1970); **75**, 62341u (1971).
[13] K. P. Boebel and D. H. Baker, *J. Nutr.* **112**, 367–376 (1982).
[14] D. H. Baker and K. P. Boebel, *J. Anim. Sci.* **53**, 125–129 (1981).
[15] G. L. Czarnecki and D. H. Baker, *J. Anim. Sci.* **55**, 1405–1410 (1982).
[16] G. F. Russell and J. I. Hills, *Science* **172**, 1043 (1971).
[17] L. Friedman and J. G. Miller, *Science* **172**, 1044 (1971).
[18] C. Günther and A. Mosandl, *Liebigs Ann. Chem.* 2112–2122 (1986).
[19] A. Gautier, C. Vail, C. Morel, M. Lander and F. Näf, *Helv. Chim. Acta* **70**, 2039–2044 (1987).
[20] M. Simonyi, *Med. Res. Rev.* **4**, 359–413 (1984).
[21] M. Simonyi, I. Fitos and J. Visy, *Trends Pharmac. Sci.* **7**, 112–116 (1986).
[22] B. Testa, *Trends Pharmac. Sci.* **7**, 60–64 (1986).
[23] T. Walle and U. K. Walle, *Trends Pharmac. Sci.* **7**, 155–158 (1986).
[24] D. F. Smith, *Prog. Neuro-Psychopharmac. Biol. Psychiat.* **8**, 327–350 (1984).
[25] N. C. Cohen, *Adv. Drug Res.* **14**, 42–145 (1985).
[26] E. J. Ariens, *Med. Res. Rev.* **6**, 451–466 (1986).
[27] E. J. Ariens, *Med. Res. Rev.* **7**, 367–387 (1987).
[28] E. J. Ariens, *Med. Res. Rev.* **8**, 309–320 (1988).
[29] W. E. Müller and U. Wollert, *Mol. Pharmac.* **11**, 52–60 (1975).
[30] T. Alebic-Kolbah, F. Kajfez, S. Rendic, V. Sunjic, A. Konowal and G. Snatzke, *Biochem. Pharmac.* **28**, 2457–2464 (1979).
[31] I. Fitos, M. Simonyi, Zs. Tegyey, L. Ötvös, J. Kajtar and M. Kajtar, *J. Chromatogr.* **259**, 494–498 (1983).
[32] I. Fitos, Zs. Tegyey, M. Simonyi, I. Sjöholm, I. Larson and C. Langercrantz, *Biochem. Pharmac.* **35**, 263–265 (1986).
[33] D. P. Craig and D. P. Mellor, *Topics Curr. Chem.* **63**, 1–48 (1976).
[34] E. J. Ariens, *Eur. J. Clin. Pharmac.* **26**, 663–668 (1984).
[35] G. Consiglio, P. Pino, L. T. Flowers and C. U. Pittman Jr, *J. Chem. Soc., Chem. Commun.* 612 (1983).
[36] M. L. Anson, K. Bailey and J. T. Edsall (Eds) *Advances in Protein Chemistry.* Academic Press, New York (1954).
[37] A. Kleemann and J. Martens, *Liebigs Ann. Chem.* 1995–1998 (1982).
[38] A. C. Mehta, *J. Chromatogr.* **426**, 1–13 (1988).
[39] D. W. Armstrong and S. M. Han, *CRC Crit. Rev. Anal. Chem.* **19**, 175–224 (1988).
[40] S. G. Allenmark, *Chromatographic Enantiomeric Separation: Methods and Applications.* Ellis Horwood, Chichester (1988).
[41] S. C. Chang, E. Gil-Av and R. Charles, *J. Chromatogr.* **289**, 53 (1984).
[42] W. A. Koenig, S. Sievers and I. Benecke, in *Proceedings of the International Symposium on Capillary Chromatography*, (R. E. Kaiser, Ed.), 4th edition, pp. 703. Inst. of Chromatography, Bad Dürkheim, FRG (1981).
[43] N. Oi, *Bunseki Kagaku* **33**, E401 (1984).
[44] R. H. Liu and W. W. Ku, *J. Chromatogr.* **271**, 309 (1983).
[45] W. A. Koenig, *High Resol. Chromatogr., Chromatogr. Commun.* **5**, 588 (1982).
[46] U. Beitler and B. Feibush, *J. Chromatogr.* **123**, 149 (1976).
[47] R. Dappen, H. Arm and V. R. Meyer, *J. Chromatogr.* **373**, 1 (1986).
[48] K. Günther, *J. Chromatogr.* **448**, 11–30 (1988).
[49] J. Martens and R. Bhushan, *Chem.-Ztg* **112**, 367–378 (1988).
[50] R. Bhushan, *J. Liq. Chromatogr.* **11** (1988). In press.
[51] F. P. Novak and D. M. Hercules, *Anal. Lett.* **18A**, 503 (1985).
[52] U. A. Th. Brinkman, *J. Planar Chromatogr.* **1**, 150–160 (1988).
[53] S. F. Contractor and J. Wragg, *Nature* **208**, 71–72 (1965).
[54] S. Yuasa, A. Shimada, M. Isoyama, T. Fukuhara and M. Itoh, *Chromatographia* **21**, 79–82 (1986).
[55] S. Yuasa, A. Shimada, M. Isoyama, T. Fukuhara and M. Itoh, *J. Chromatogr. Sci.* **18**, 311 (1980).
[56] V. A. Davankov, A. S. Bochkov and Yu. P. Belov, *J. Chromatogr.* **218**, 547–557 (1981).
[57] V. A. Davankov, A. S. Bochkov, A. A. Kurganov, P. Roumeliotis and K. K. Unger, *Chromatographia* **13**, 677–685 (1980).
[58] J. Martens, H. Weigel, E. Busker and R. Steigerwald, DE-PS 3143726, Degussa AG, Hanau, FRG.
[59] K. Günther, J. Martens and M. Schickedanz, *Angew. Chem.* **96**, 514–515 (1984).
[60] K. Günther, J. Martens and M. Schickedanz, *Angew. Chem. Intl. Ed. Engl.* **23**, 506 (1984).
[61] J. Martens, K. Günther and M, Schickedanz, *Arch. Pharm. (Weinheim)* **319**, 572–574 (1986).
[62] K. Günther, J. Martens and M. Schickedanz, *Fresenius Z. Anal. Chem.* **322**, 513–514 (1985).
[63] J. Martens, K. Günther and M. Schickedanz, *Arch. Pharm. (Weinheim)* **319**, 461–465 (1986).
[64] K. Günther, J. Martens and M. Schickedanz, *Naturwissenschaften* **72**, 149–150 (1985).
[65] K. Günther and R. Rauch, *Proceedings of the Third International Symposium on Instrum. HPTLC*, Würzberg, pp. 469–476. Inst. for Chromatography, Bad Dürkheim (1985).
[66] K. Günther, J. Martens and M. Schickedanz, *Angew. Chem.* **98**, 284–285 (1986); *Angew. Chem. Intl. Ed. Engl.* **25**, 278–279 (1986).
[67] K. Günther, M. Schickedanz, K. Drauz and J. Martens, *Fresenius Z. Anal. Chem.* **325**, 297–298 (1986).
[68] K. Günther, *GIT Suppl* **3**, 6–12 (1986).
[69] U. A. Th. Brinkman and D. Kamminga, *J. Chromatogr.* **330**, 375–378 (1985).
[70] B. K. Vriesema, W. tenHoeve, H. Wynberg, R. M. Kellog, W. H. J. Boesten, E. M. Meijer and H. E. Shoemaker, *Tetrahedron Lett.* **26**, 2045–2048 (1986).
[71] R. Bhushan and I. Ali, *Chromatographia* **23**, 141–142 (1987).
[72] H. Brückner, I. Bosch, T. Graser and P. Furst, *J. Chromatogr.* **395**, 569–590 (1987).

[73] E. Neuzil, A. M. Lacoste and F. Denois, *Biochem. Soc. Trans.* **14**, 642–643 (1986).
[74] L. K. Gont and S. K. Neundorf, *J. Chromatogr.* **391**, 342–345 (1987).
[75] H. Jork and E. Kany, GDch-Fortbildungkurs No. 301, Saarbrücken (1986).
[76] R. Klaus and W. Fischer, *Chromatographia* **23**, 137–140 (1987).
[77] M. L. Bender and M. Komiyama, *Cyclodextrin Chemistry*. Springer, Berlin (1978).
[78] W. L. Hinz, *Sep. Purif. Meth.* **10**, 159 (1981).
[79] D. W. Armstrong, *J. Liq. Chromatogr.* **3**, 895 (1980).
[80] W. L. Hinze and D. W. Armstrong, *Anal. Lett.* **13**, 1093 (1980).
[81] A. Alak and D. W. Armstrong, *Anal. Chem.* **58**, 582 (1986).
[82] S. Weinstein, *Tetrahedron Lett.* **25**, 985–986 (1984).
[83] N. Grinberg and S. Weinstein, *J. Chromatogr.* **303**, 251–255 (1984).
[84] R. Marchelli, R. Virgilli, E. Armani and A. Dossena, *J. Chromatogr.* **355**, 354–357 (1986).
[85] M. Sinibaldi, A. Messina and A. M. Grelli, *Analyst (Lond.)* **113**, 1245–1247 (1988).
[86] R. Bhushan and I. Ali, *J. Chromatogr.* **392**, 460–462 (1987).
[87] K. T. Wang, S. T. Shen and L. C. Lo, *Fresenius Z. Anal. Chem.* **324**, 339–340 (1986).
[88] H. Spahn, *J. Chromatogr.* **427**, 131–137 (1988).
[89] R. J. Raune and I. D. Wilson, *J. Chromatogr.* **441**, 355–360 (1988).
[90] R. S. Feldberg and L. M. Reppuci, *J. Chromatogr.* **410**, 226–229 (1987).
[91] K. Günther and M. Schickedanz, *GIT Suppl* **3**, 27–32 (1987).

[*Received for review 31 October 1988; revised manuscript received 2 May 1989*]