Journal of Pharmaceutical & Biomedical Analysis
Vol. 6, Nos 6–8, pp. 641–656, 1988
Printed in Great Britain

0731–7085/88 $3.00 + 0.00

Chiral stationary phases for the liquid chromatographic separation of pharmaceuticals*

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Abstract: Biological systems exhibit remarkable enantioselectivity which is important in biosynthesis, metabolism, storage and transport processes. It is, therefore, not surprising that the chirality of, for example, pharmaceuticals, pesticides and agrochemicals, has become the focus of extensive research in many laboratories throughout the world. Chiral liquid chromatography is currently very popular for enantiomeric separations since the selectivities afforded by a wide range of commercially available chiral phases enable the separations of many classes of compounds to be routine. This paper discusses the most important types of chiral phases currently in use and reviews their applications to the analysis of compounds of pharmacological interest.

Keywords: *Enantiomers; chiral stationary phases; pharmaceuticals; classification of chiral columns.*

Introduction

Since the first separation of the racemic mixture of sodium ammonium tartrate by Louis Pasteur in 1848 [1], enantiomeric separations have been considered as one of the most difficult problems in separation science.

Enantiomers are stereoisomers which rotate plane-polarized light in the opposite direction with respect to each other; they are non-superimposable mirror images. Since enantiomers differ only in the absolute spatial arrangement, they have identical internal energies and, thus, physical and chemical properties in an isotropic environment. Stereoisomers which are not enantiomers (i.e. they are not mirror images) are called *diastereomers*. Unlike enantiomers, diastereomers have different physical properties and can be resolved on a non-chiral system.

Many therapeutic agents used today contain asymmetric carbon, phosphorus, nitrogen or sulfur atoms. It is estimated that approximately one half of the 700 most frequently prescribed drugs contain a chiral centre and approximately one half of these are marketed as racemic mixtures [2].

It has been said that "plasma protein binding can function as a biological machine that performs what pharmaceutical companies often neglect namely the resolution of drugs

*Presented at the "International Symposium on Pharmaceutical and Biomedical Analysis", September 1987, Barcelona, Spain.

into enantiomers" [3]. Enantiomers should be regarded as different chemical compounds in a biological medium because they may show considerable differences in potency, type of pharmacological activity, pharmacokinetic profile and metabolism since the majority of the enzymatically catalysed reactions are stereoselective. *In vivo* interconversion of enantiomers and thus a possible loss or accumulation of the active component can also be envisaged. Therefore a racemic drug may be regarded as a mixture containing 50% or more of an inactive isomer which is presumed to be harmless.

The importance of enantiomeric purity is best exemplified by the thalidomide (Neurosedyn) catastrophe where the S(−) isomer was responsible for the teratogenic effect and the R (+) form for the sleep-inducing effect [4]. This example demonstrates the importance of studying single enantiomers in order to optimize therapeutic usefulness and minimize adverse effects. The choice between a racemate and a pure enantiomer of pharmacological interest is thus dictated by the extent of side effects, differences in potency and toxicity and economic reasons.

Methods for analytical and preparative resolution of racemic compounds are being developed in parallel to the asymmetric synthesis. It should be emphasized that these two approaches are not mutually exclusive, since in cases where the synthesis is not 100% stereospecific both techniques may be needed. Prior to the development of gas and liquid chromatographic methods, enantiomeric mixtures were traditionally resolved by (a) *fractional crystallization* of diastereomeric derivatives formed by the reaction between an optically active agent (chiral selector) and the enantiomeric solutes (chiral selectand) (b) *microbiological digestion* or (c) *enzymatic digestion*. These methods are still used both on analytical and preparative scales because it is sometimes easier, cheaper and faster to chemically resolve a racemate than to develop an enantioselective chromatographic technique. However, the most important of these methods, the separation via formation of diastereomers suffers from three major drawbacks, namely the need for chiral reagents of high enantiomeric purity, kinetic problems due to different reaction rates for the two enantiomers, and possible racemization of the chiral centres either in the solute or in the chiral reagent. In addition, the solute molecule must possess a functional group amenable to derivatization.

Since the mid-1960s, researchers have been developing chiral phases for chromatographic resolution of enantiomers. Initially, gas chromatography (GC) using chiral stationary phases or solute derivatization to form disatereomers seemed promising. However, the need for optically pure and thermally stable derivatization reagents, the possibility of racemization either of the chiral phase or the analyte at higher temperatures, and diminished stability differences (for entropic reasons), and thus the extent of the separation between the diastereomeric adsorbates, limit the use of this method. In addition, preparative separations by GC are generally not feasible.

Although the development of LC chiral separations began in the 1970s, the widespread commercialization of chiral stationary phases and the development of new chiral mobile phase additives began in the early 1980s. There are two general approaches to the direct LC separation of chiral compounds. (a) the use of *chiral stationary phases and achiral mobile phases* and (b) *the use of achiral stationary phases in conjunction with chiral mobile phase additives*. Both types of methods rely on the temporary formation of short-lived diastereomeric adsorbates of different stabilities, where the more stable one is preferentially retained.

Literature examples of chiral mobile phase additives include the use of chiral bidentate ligands and transition metal ions such as Cu(II), Zn(II), Cd(II), etc. [5–9]. This method

is a variation of chiral ligand-exchange LC. Chiral ion-pairing agents such as (+)-10-camphorsulfonic acid [10], tartaric acid derivatives [11], proteins [12] or chiral additives such as cyclodextrins [13] have also been used. These methods sometimes require costly additives which may have to be tailored for a particular application. Furthermore, they must be removed from the analyte in preparative applications.

Chiral stationary phases (CSPs)

At present, there is no chiral bonded phase capable of separating all classes of compounds, so that analysts must choose the right phase for a particular application from, at present, 31 commercially available chiral columns. Since most chiral columns are expensive compared with conventional columns, it is important to select the most suitable phase to avoid unnecessary expense. With a better understanding of the different mechanisms of chiral recognition and an increasing number of applications, the classification of phases according to the types of interactions involved and the establishment of general guidelines for the choice of a column based on molecular structure, become essential. This subject has recently been treated by Wainer [14].

The first model for chiral recognition in chromatography was developed by Dalgliesh in 1952 [15]. The basis of this model is the "three-point interaction" between the solute and the CSP. This simplified model of chiral recognition originates from the Cahn–Ingold–Prelog convention according to which at least three of the four bonds of the chiral carbon atom are needed to determine the configuration. According to this model, at least three of the stereochemical elements of the solute and CSP must be involved in the chiral recognition model [14]. The interactions between the chiral selector and select and, which may be either attractive or repulsive, involve *hydrogen bonding*, *dipole–dipole*, π–π, *electrostatic*, *hydrophobic* or *steric* interactions.

However, if one interaction defines an axis rather than a point (e.g. interactions between two aromatic rings), only two interactions may be sufficient. The highest degree of enantioselectivity will be obtained when the three interaction points (one of which must be stereochemical) are close to the asymmetric centre. Enantioselectivity decreases with increasing distance between the chiral centre and the interaction groups because of the smaller difference between the average conformations of the temporary diastereomeric complexes.

Generally speaking, the interactions responsible for retention and separation of enantiomers can serve as a basis for the classification of CSPs into four categories (Table 1): Pirkle-type phases (I), chiral polymers (II), affinity phases (III) and chiral ligand-exchange phases (IV). The distinction between types I and II phases is based on the difference of location of the interactive sites.

CSPs type I

These phases have been developed by Pirkle *et al.* [16–17]. Hydrogen bonding, π–π interactions, dipole stacking, and electrostatic attraction are operative on these phases. They are prepared by reacting the α-amino group of (R)-phenylglycine, (S)-phenylglycine (S)-leucine or (R)- and (S)-naphthylalanine or (S)-naphthylvaline with 3,5-dinitrobenzoyl chloride. The latter reagent is responsible for π–π interactions and the amide bond for hydrogen bonding and dipole stacking interactions. The product of this reaction is then ionically or covalently bonded to the aminopropyl functionalized silica. They are commonly used with non-aqueous mobile phases composed of hexane and an alcohol as a polar modifier.

Table 1
Commercially available HPLC enantioselective stationary phases

Chiral phase	Supplier	Mobile phase used	Applications
Type I (Pirkle-type phases)			
(R)-N-(3,5-Dinitrobenzoyl)phenylglycine (covalent)	Regis, J. T. Baker	hexane–isopropanol	Aromatic sulfoxides; 3,5-dinitrobenzoyl derivatives of amines, alcohols, thiols amino acids, amino alcohols and hydroxy acids; aryl-substituted lactams, succinimides, hydantoins, hydroxylphosphonates; oxazolidones binaphtols; drug derivatives
(R)-N-(3,5-Dinitrobenzoyl)phenylglycine (ionic)	Regis, J. T. Baker	hexane–isopropanol	
(S)-N-(3,5-Dinitrobenzoyl)phenylglycine (covalent)	Regis	hexane–isopropanol	
(S)-N-(3,5-Dinitrobenzoyl)leucine (covalent)	Regis, J. T. Baker	hexane–isopropanol	Aromatic sulfoxides; 3,5-dinitrobenzoyl derivatives of amines, alcohols, thiols amino acids, amino alcohols and hydroxy acids; aryl-substituted lactams, succinimides, hydantoins, hydroxylphosphonates; oxazolidones binaphtols; drug derivatives
(S)-N-(3,5-Dinitrobenzoyl)leucine (ionic)	Regis, J. T. Baker	hexane–isopropanol	
D-Naphthlalanine	Regis	hexane–isopropanol	Dinitrobenzoyl derivatized compounds
L-Naphthylalanine	Regis	hexane–isopropanol	Dinitrobenzoyl derivatized compounds
(R)-α-Methylbenzylurea	Supelco	hexane–isopropanol	
(R)-(+)-Naphthylethylamine polymer	YMC	hexane–isopropanol	3,5-Dinitroanilide der. of carboxylic acids, 3,5-dinitrobenzoyl der. of amino acids
1-(α-Naphthyl)ethylamine derivative	Sumitomo	hexane–isopropanol	
(S),(S)-α-Naphthylethylaminocarbonylvaline	Sumitomo	hexane–isopropanol	3,5-Dinitrobenzoyl derivat. of amino acid esters, amines and amides, carboxylic acids
(R),(R)-α-Naphthylethylaminocarbonylvaline	Sumitomo	hexane–isopropanol	
Type II (polymeric polymeric phases) — A			
Cellulose triacetate	Daicel, J. T. Baker	hexane–isopropanol	Sulfoxide derivatives of organophosphorus compounds and insecticides, barbiturates, cyclic thioamides, disubstituted cyclic racemates, β-adrenergic blocking agents
Cellulose tribenzoate	Daicel, J. T. Baker	hexane–isopropanol	
Cellulose triphenylcarbamate	Daicel, J. T. Baker	hexane–isopropanol	
Cellulose tricinnamate	Daicel, J. T. Baker	hexane–isopropanol	
Cellulose tris(3,5-dimethylphenylcarbamate)	Daicel, J. T. Baker	hexane–isopropanol	

— B			
Poly(triphenylmethylmethacrylate)	Daicel, J. T. Baker	méthanol	Binaphthalene derivatives, cyclic insecticides, transdisubstituted racemates, bisazulenes, derivat. glucitol
β-Cyclodextrin	Advanced separation technologies	aqueous buffers acetonitrile/methanol	Dansyl and naphthyl amino acids, several aromatic drugs, steroids, alkaloids metallocenes, binaphthyl crown ethers, aromatic acids, aromatic amines, aromatic sulfoxides
α-Cyclodextrin	Advanced separation technologies	aqueous buffers acetonitrile/methanol	Barbiturates, aromatic amino acids
γ-Cyclodextrin	Advanced separation technologies	aqueous buffers acetonitrile/methanol	Stereoisomers of polycyclic aromatic hydrocarbons
Type III (affinity phases)			
Bovine serum albumin	Anspec, Macherey-Nagel	Aqueous buffers	Cyclic and aromatic drugs
α-Acid glycoprotein	LKB	Aqueous buffers	N-benzoyl amino acids, aromatic sulfoxides, some drugs
Type IV (ligand exchange)			
Proline	Daicel, J. T. Baker	Aqueous buffer-Cu(II)	Underivatized and some derivatized amino acids
Hydroxyproline	Machery-Nagel Serva	Aqueous buffer-Cu(II)	Underivatized and some derivatized amino acids
Valine	Serva	Aqueous buffer-Cu(II)	Underivatized and some derivatized amino acids

Quite often, derivatization of the solute is required since these phases usually impose strict structural requirements [18]: amines are usually converted to amides [19] or carbamates [20] and carboxylic acids to esters [21] or amides [19]. Compounds with α, β-amino alcohol functionalities are often separated after conversion to the corresponding oxazolidone [22] and oxazolidine derivatives [23].

In order to maximize the π–π interactions, the most commonly used derivatization reagents contain a naphthyl moiety (when 3,5-dinitrobenzoyl CSPs are used) or a 3,5-dinitrobenzoyl moiety (when naphthylalanine CPSs are used).

Relatively little information is available on the use of these columns for the separation of drugs [24, 25]. Wainer *et al.* [24] have investigated the use of the (R)-*N*-(3,5-dinitrobenzoyl)phenylglycine-bonded phase for the enantiomeric resolution of α-methylarylacetic acid anti-inflammatory agents used for the treatment of acute and chronic rheumatoid arthritis and osteoarthritis.

These separations are of interest since it is known that their (R)-(−)-enantiomers were found to be converted *in vivo* into the corresponding (S)-(+) enantiomers. These separations are illustrated with the separation of the enantiomers of the amide derivative of ibuprofen. Figure 1 illustrates the postulated orientations and conformations for optimal interaction between the (R)-ibuprofen amide and the CSP:hydrogen bonding between the amide dipoles of the CSP and the solute molecule, the π–π interactions between the aromatic ring of ibuprofen and the phenyl ring of the phenylglycine moiety of the CSP and σ–π or π–π interactions between the R group of the amide and the 3,5-dinitrophenyl ring of the CSP are operative only in the (R)-isomer in which the α-methyl group is on the top of the solute-CSP complex. In the (S)-isomer this group interferes with optimal interaction, thus it is eluted before the (R)-isomer (Fig. 2).

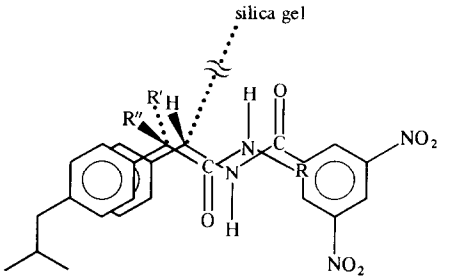


Figure 1
A chiral recognition model describing the interaction between an amide and the Pirkle CSP. (S)-enantiomer of ibuprofen amide, R′ = CH_3, R″ = H; (R) enantiomer of ibuprofen amide, R′ = H, R″ = CH_3. For the sake of clarity, the relative positioning of the amide and CSP is shifted slightly from the best overlap. (Reproduced from Ref. [25] with permission.)

CSPs type IIA

In 1980, Blaschke described the use of cellulose, starch and cellulose acetate as chiral sorbents [26]. Recently, some new promising phases were developed by Okamoto's group [27]. They are cellulose triesters coated on macroporous silica (Table 1). They belong to the general category of polysaccharide sorbents and the formation of diastereomeric complexes involves hydrogen bonding, π–π and dipole interactions. These interactive sites are located within the cavities, rather than on the surface as in CSPs type I. These phases can be used with mobile phases composed of a non-polar solvent (hexane) modified with an alcohol or with polar eluents (e.g. ethanol); chlorinated solvents should be avoided.

The crystallinity of the cellulose was shown not to be critical for chiral recognition [28]; however, the molecular mass of the cellulose derivative, the solvent used for depositing

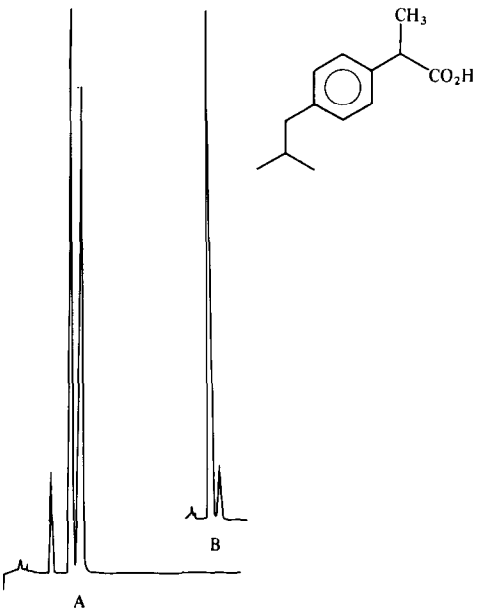


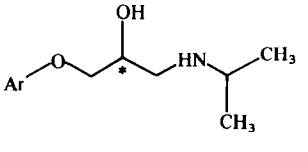
Figure 2
Representative chromatogram showing the separation of the enantiomers of ibuprofen amide (A) racemic mixture, (B) 91:9 (S) to (R) mixture. Chromatographic conditions: Pirkle column (R)-*N*-(3,5-dinitrobenzoyl)phenylglycine (covalent); mobile phase; hexane-propan-2-ol (97:3, v/v); flow-rate: 2 ml min^{-1}; UV detection at 254 nm. (Reproduced from Ref. [25] with permission.)

the phase on the support, and the nature of the support were found to have an effect. Most compounds successfully separated on these phases contain a phenyl, carbonyl, nitro, cyano, sulfonyl, sulfinyl or hydroxyl groups. Compounds with polar groups such as carboxylic acids and primary amines are usually derivatized prior to chromatography. Among examples of drug separations on these phases, the most important ones are those of β-adrenergic blocking agents separated on tris(3,5-dimethylphenylcarbamate)-cellulose [29, 30].

β-Adrenergic blocking agents used in the treatment of hypertension contain in their general formula **1** a chiral centre in position **2** (*) (Table 2).

Although many of these drug substances are developed and marketed as a racemic mixture of both enantiomers, for some of them only one enantiomer, usually the S-isomer is preferred [31], so that it is important to assess their enantiomeric purity. Figure 3 illustrates the separations of some commonly used β-adrenergic blocking agents prescribed for the treatment of hypertension (Table 2).

In the synthesis of 1-{4-[2-(cyclopropylmethoxy)ethyl]phenoxy}-3-isopropylamino-2-propanol hydrochloride, the (S)-(−)-enantiomer of a β-blocker (Kerlone®) developed by our company for use in hypertension and by Alcon for the treatment of glaucoma (Betoptic®), the stereochemistry of the final product is fixed in one of the early stages of the synthetic route. Subsequent reactions do not generate optically labile intermediates



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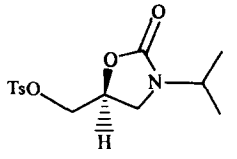

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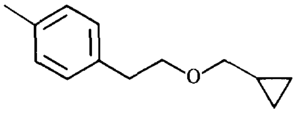
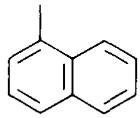
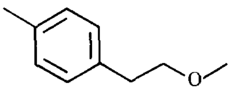
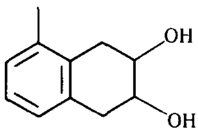
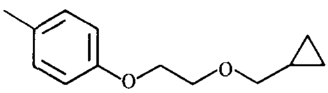
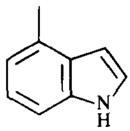
Table 2

$$ArOCH_2\underset{\displaystyle OH}{\overset{*}{C}}HCH_2NHCH(CH_3)_2$$

Name	Ar	R_s
Betaxolol		2.1
Propranolol		1.0
Metoprolol		1.1
Nadolol		0.97
Cicloprolol		1.7
Pindolol		5.1

or provide conditions for facile partial or total racemization. Thus in-process control of chiral intermediate 2 assures high enantiomeric purity of the final product. Figure 4(A) shows the chromatogram of a racemic mixture of the intermediate, (B) the (S)-enantiomer of the intermediate and (C) the final product. The effect of the polarity of the organic modifier on the resolution of the enantiomers of betaxolol (Kerlone®) separated on the Chiralcel OD column is shown in Fig. 5.

CSPs type IIB

This group of chiral phases comprises β- and γ-cyclodextrins and the synthetic polymers such as poly(triphenylmethylmethacrylate) and poly(2-pyridyldiphenyl-

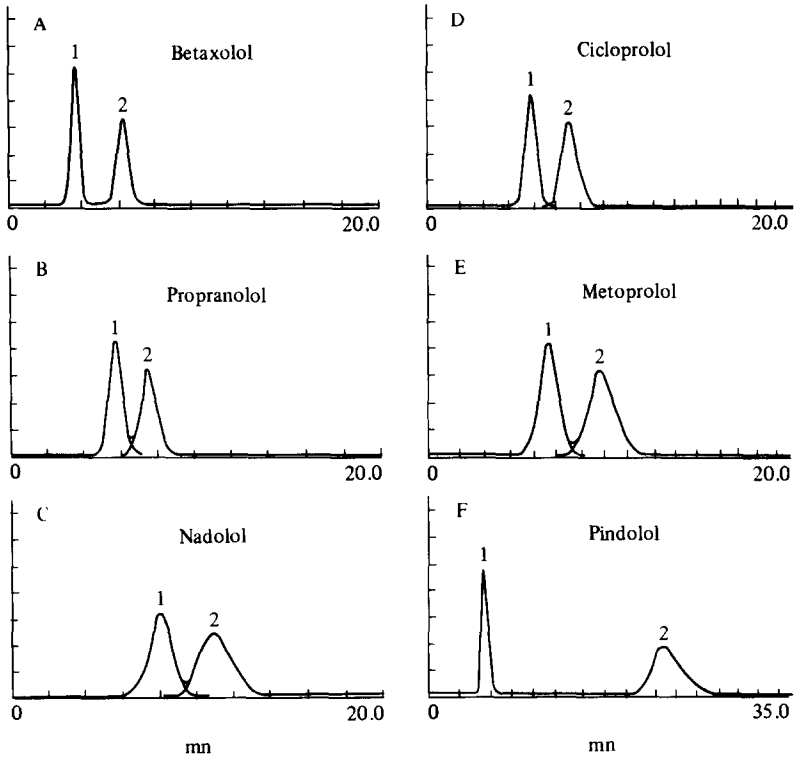


Figure 3
Examples of separations of enantiomers of β-adrenergic blocking agents on a Chiralcel OD column. (A) *betaxolol*; chromatographic conditions: mobile phase: hexane-propan-2-ol (83:17, v/v); flow-rate: 1.5 ml min^{-1}. (B) *propanolol*; chromatographic conditions: mobile phase: hexane-propan-2-ol-ethanol (80:5:15, v/v/v); flow-rate: 1.0 ml min^{-1}. (C) *nadolol*; conditions as in (B). (D) *cicloprolol*; conditions as in (B). (E) *metoprolol*; chromatographic conditions: mobile phase: hexane-ethanol (90:10, v/v); flow-rate: 1.0 ml min^{-1}. (F) *pindolol*; chromatographic conditions: mobile phase: hexane-propan-2-ol (70:30, v/v); flow-rate: 1.5 ml min^{-1}. Column temperature was 22 ± 1°C in all cases.

methylmethacrylate) (Table 1). The chiral recognition afforded by these phases is based on the inclusion of the solute molecules in the chiral cavity of the stationary phase.

Cyclodextrins (CD) are cyclic oligosaccharides composed of 6 to 8 D-glucose units linked together through α-(1,4) linkages. The molecule has the shape of a hollow truncated cone with a maximum diameter varying from 5.7 Å (α-CD) to 9.5 Å (γ-CD) with a depth of 7 Å and contains 30–40 chiral centres for enantiomeric selectivity. The larger opening of the cone is rimmed with 14 secondary 2-hydroxy groups, the smaller opening is rimmed with 7 more polar primary hydroxyl groups, while the interior is relatively hydrophobic. Armstrong and collaborators developed a material suitable for use in HPLC using cyclodextrin bonded to silica via a 6- or 10-atom spacer [31, 32]. The separation of the enantiomers requires a tight fit of the less polar (preferably aromatic) portion of the molecule into the cavity. Three types of cyclodextrins are commercially available: α-cyclodextrin with six glucose units (cavity i.d. = 5.7 Å), β-cyclodextrin containing seven glucose units (cavity i.d. = 8 Å), and γ-cyclodextrin with eight glucose units (cavity i.d. = 9.5 Å).

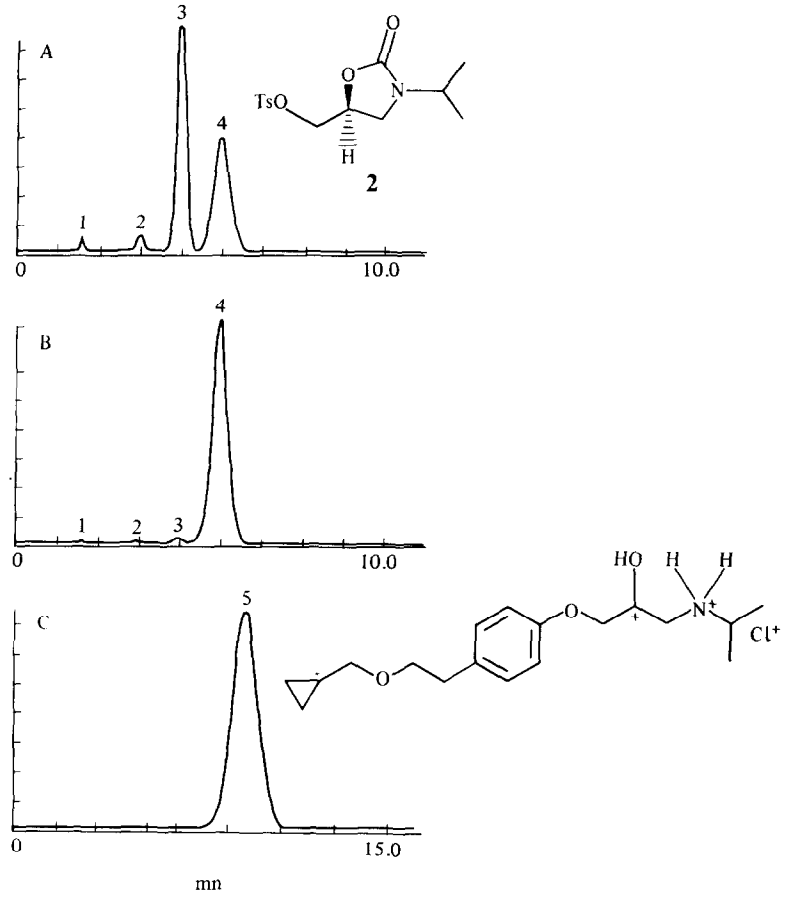


Figure 4
(A) Chromatogram illustrating the separation of the enantiomers of (5R,S)-3-isopropyl-5-*p*-toluene-sulfonyloxymethyloxymethyl-oxazolidin-2-one. Chromatographic conditons: column: Chiralcel OB (250 × 4.6 mm i.d.); mobile phase: methanol; flow-rate: 1.0 ml min^{-1} column temperature: 22 ± 1°C. (B) In-process control of enantiomeric purity of (5S)-3-isopropyl-5-*p*-toluenesulfonylmethyloxazolidin-2-one, an intermediate used in the synthesis enantiomerically pure S-betaxolol. Chromatographic conditions as in (A). (C) Determination of the enantiomeric purity of S-betaxolol; chromatographic conditions: column: Chiralcel OD (250 × 4.6 mm i.d.); mobile phase: hexane-propan-2-ol (85:15, v/v); flow-rate: 1 ml min^{-1}. Column temperature: 22 ± 1°C.

There are few applications of α- and γ-cyclodextrin phases in the literature. β-cyclodextrin phase has been successfully used for the separation of the enantiomers of dansyl amino acids, β-naphthylamide, β-naphthyl esters of amino acids, and barbiturates [33, 34]. These phases are used with buffered hydro-organic eluents containing methanol, ethanol or acetonitrile as the organic modifier. Preparative separations are also possible. When used with non-polar eluents, these phases are achiral and give selectivities similar to the diol phases. This is due to the solvent competition for the hydrophobic interior of the cavity.

The use of these phases is illustrated with the analysis of rat plasma levels of enantiomers of an experimental drug recently developed by our company (Scheme 1).

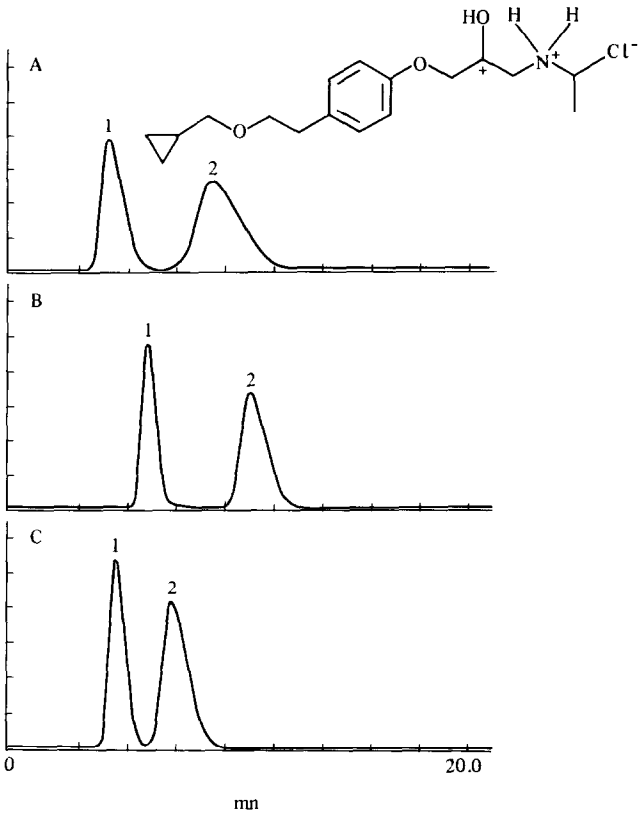
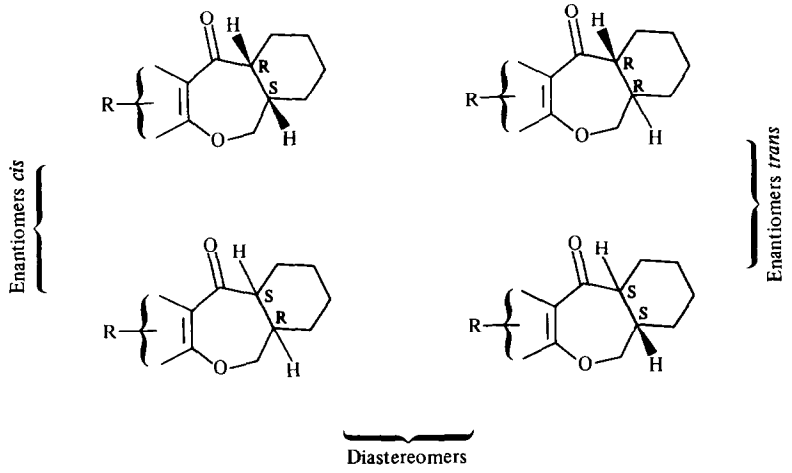


Figure 5
The influence of the organic modifier on the separation of the enantiomers of betaxolol. Chromatographic conditions: column Chiralcel OD (250 × 4.6 mm i.d.); mobile phase: (A) hexane-*n*-butanol (85:15, v/v); flow-rate: 1.0 ml min^{-1}; Rs = 1.7. (B) hexane-propan-2-ol (85:15, v/v); flow-rate: 1.0 ml min^{-1}; Rs = 2.7. (C) hexane-ethanol (92:8, v/v); flow-rate: 1.0 ml min^{-1}; Rs R 1.5. Column temperature: 22 ± 1°C.



Scheme 1

The compound has two asymmetric centres, each of which can have the absolute configuration R or S; in the absolute configurations R–R or S–S, the two hydrogens are *trans*, while in the configuration R–S or S–R they are *cis*. In the compound under study, the configuration is *trans*, as confirmed by H^1NMR and circular dichroism (CD). Figure 6(a) shows the separation of a reference racemic mixture and (b) and (c) the enantiomers in a dichloromethane extract of rat plasma 3 h and 6 h after administration of the drug. It is evident from the figure that the (−)-enantiomer is metabolized faster than the (+)-enantiomer.

The poly(triphenylmethylmethacrylate) phases recently reviewed by Okamoto *et al.* [35] are prepared by asymmetric polymerization of triphenylmethylmethacrylate with chiral anionic initiators. They represent an interesting class of vinyl polymers whose stereoselectivity arises only from the helicity of the polymer due to the orientation of the triphenyl groups. These columns are used with pure methanol or mixtures of hexane and propan-2-ol. Certain organic solvents such as chloroform, THF or aromatic hydrocarbons are excluded since they dissolve the polymer.

CSPs type III

These phases utilise the stereo-differentiating properties of proteins (Table 1). Currently, the two most useful chiral protein columns are human α-acid glycoprotein (AGP) originally developed by Hermansson [36] and bovine serum albumin (BSA) developed by Allenmark [37]. Human AGP has a molecular mass of approximately 40,000 and it is composed of a 181-unit peptide chain representing 55% of the molecular mass and five carbohydrate units which comprise the remainder. These latter moieties

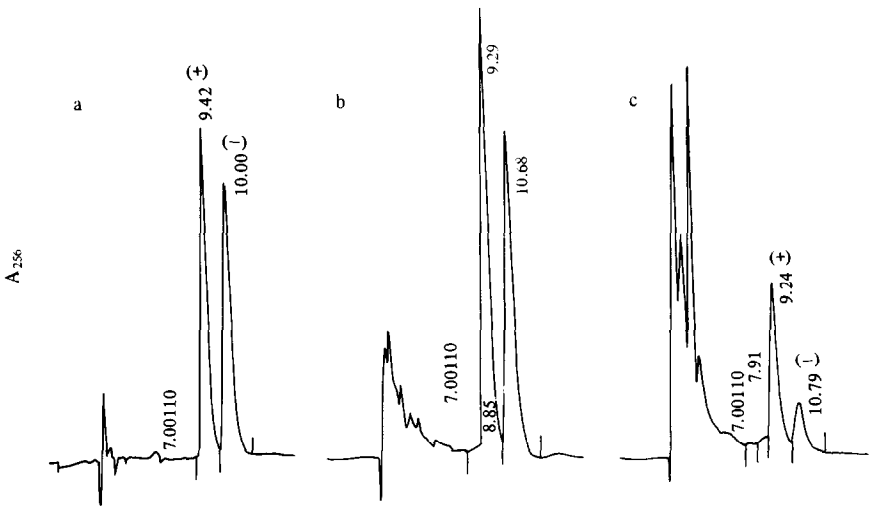


Figure 6
(a) Chromatogram of a racemic mixture of enantiomers of the compound shown in Scheme 1. Chromatographic conditions: column: Cyclobond I (β), 10 μm (250 × 4.6 mm i.d.); mobile phase: 0.05 M KH_2PO_4, (pH 5.3) — methanol (35:65, v/v); flow-rate: 1.0 ml min^{-1}, column temperature: 22 ± 1°C; detection: UV absorbance at 254 nm. (b) Chromatogram of a dichloromethane extract of a 1.0-ml sample of rat plasma analyzed under the same conditions as in (a). Blood sample was withdrawn 3 h after oral administration of the drug. (c) Chromatogram of a dichloromethane extract of a 2.0-ml sample of rat plasma analysed under the same conditions as in (a). Blood sample was withdrawn 6 h after oral administration of the drug.

contain 141 sialic acid residues. The isoelectric point of AGP is 2.7 in phosphate buffer. Since a number of carboxylic acid moieties are utilized in binding the protein to the silica, the stereospecificity of the protein is somewhat modified with respect to the native protein.

BSA is a globular protein (molecular mass 66,210) consisting of 581 amino acids with 17 interchain disulpide bridges which connect 34 half-cystines with the formation of nine double loops [37]. Its isoelectric point is 4.7. At pH 7.0, its net charge is −18 and its overall character is hydrophobic.

The enantioselective properties based on principles of bioaffinity of the two proteins, involve a combination of hydrogen bonding, electrostatic interactions and hydrophobic interactions; in addition, charge-transfer interactions may be operative on BSA and ion-pairing on AGP. A prerequisite for optical resolution on BSA columns is the presence of aromatic as well as relatively polar groups in the racemic compound. Steric effects also seem to be highly important and can be responsible for some unpredictable results.

Both columns are normally used with buffered aqueous-organic eluents. Retention can be controlled by means of pH, ionic strength of the buffer, polarity of the organic modifier and addition of cationic (amines, quaternary ammonium salts) or anionic (aliphatic organic acid) additives.

These phases have been used for the separation of a variety of underivatized anionic and cationic solutes, mostly drugs. Compounds containing α, β-amino alcohols must be converted to oxazolidone derivatives prior to their separation on the AGP column. Both columns exhibit excellent selectivities but limited efficiencies and sample capacities. Preparative separations are difficult.

The use of the AGP column is illustrated with the separation of enantiomers of alfuzosine, *N*-[3-[(4-amino-6,7-dimethoxy-2-quinazolinyl)methylamino]propyl]-tetrahydro-2-furancarboxyamide hydrochloride (Fig. 7A), an antihypertensive agent developed by our company. Figure 7B shows the dramatic effect of tetrabutylammonium bromide (TBA) on the separation of the enantiomers of alfuzosine; at pH 7.4, the protein is negatively charged resulting in strong interactions with the quinazoline ring and, thus, excessively long retention times. Upon addition of TBA these interactions are diminished while a satisfactory resolution is maintained. Similarly, the addition of octanoic acid also reduces the analysis time while maintaining satisfactory resolution. Contrary to the situation with TBA which acts on the stationary phase, octanoic acid participates in ion-pair formation with the primary amino group in the alfuzosine molecule. Thus the interactions between the protonated amino groups of the solute and the negatively charged stationary phase are diminished.

CPSs type IV

Chiral ligand-exchange chromatography was pioneered by Davankov *et al.* [38] for the separation of amino acid racemates. In general, polymers such as polystyrene or polyacrylamide containing chiral chelating agents such as amino acids are loaded with Cu(II), Ni(II), Zn(II) or Cd(II). Gübitz *et al.* [39], bonded L-proline, L-hydroxyproline, L-histidine, L-phenylalanine or L-valine to silica via a 3-glycidoxipropyltrimethoxysilane spacer and loaded them with Cu(II) for the separation of amino acids and their derivatives (Table 1). The solutes must form coordination complexes with the transition metal. These phases are used with aqueous eluents containing 0.25 M Cu (II) sulfate. Amino acids, thyroid hormones, dipeptides and α-hydroxy acids have been separated on these columns.

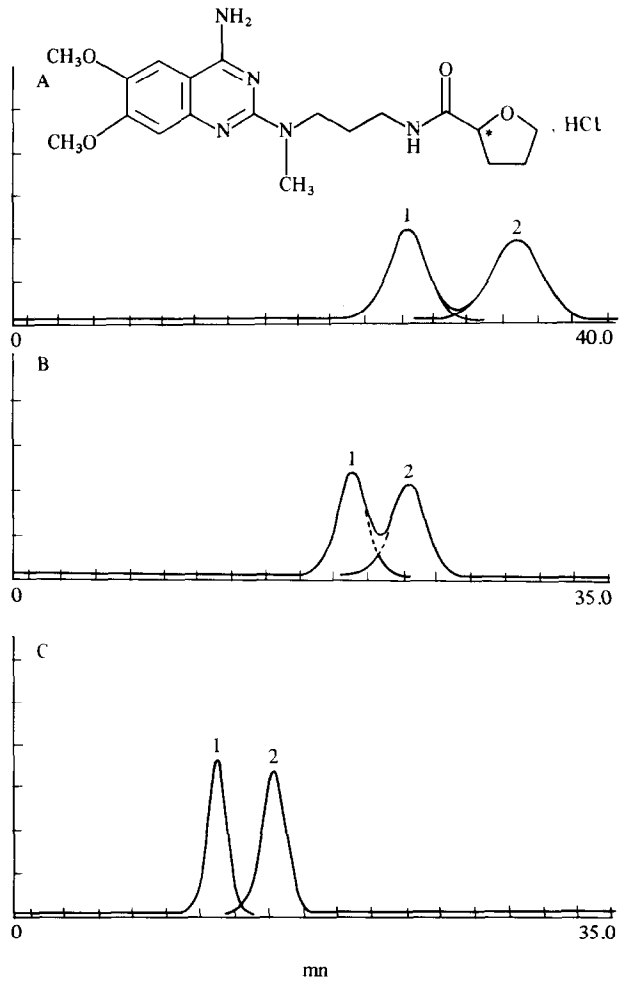


Figure 7
Separation of enantiomers of alfuzosine. Chromatographic conditions: column: EnantioPac cartridge (100 × 4.0 mm i.d.); mobile phase: (A) 0.1 M ammonium acetate, (pH 7.5)-propan-2-ol (98:2, v/v); flow-rate: 0.3 ml min^{-1}; *Rs* = 1.2. (B) 0.1 M ammonium acetate, (pH 7.5) containing 10 m mol l^{-1} octanoic acid-propan-2-ol (98:2, v/v); flow-rate: 0.3 ml min^{-1}; *Rs* = 0.8. (C) 0.1 M ammonium acetate, (pH 7.5) containing 30 mmol l^{-1} TBA-propan-2-ol (98:2, v/v); flow-rate: 0.3 ml min^{-1}; *Rs* = 1.3.

Other CPSs

There have been literature reports on several other types of CPSs which are not yet commercially available, such as those based on tartaric acid derivatives [40] and charge-transfer molecules immobilized on silica gel [41]. However, relatively little information is available on the use of these columns.

Conclusions

At present, no universal CSP exists so that as the number of commercially available chiral columns is increasing rapidly, researchers are faced with the problem of selecting

the right column for their application. The most critical step in the analysis is the choice of the right column because optimizations are restricted to fewer separation parameters than for conventional LC, due to severe limitations imposed by the manufacturers. Consequently, in most cases, selective work-up procedures must be developed for the analysis of complex matrices. Efficiencies are often low (several hundred plates per column length) which results in limited senesitivities. Equilibration times are generally long and temperature variations often have a profound effect on the separation. Most columns are rather fragile, expensive, and the column-to-column reproducibility is sometimes poor. Scale-up to preparative separations require large selectivities which are sometimes difficult to achieve. One problem which has not been extensively treated thus far is that of more specific detection devices to avoid false enantiomeric peaks. Chiroptic detectors based on circular dichroism and polarimetry which respond only to optically active solutes are being developed and commercialized. Their use will afford selectivity which is particularly needed in pharmacokinetic and metabolic studies.

Acknowledgements — The author would like to thank the Director of the Chemistry Department, Dr Alexander Wick, for his advice, the Analytical group of L.E.R.S., the students Marie-Hélène Fouchet, Jean-Marie Gianviti and Pierre Menozzi for technical assistance and Dr Jim Burke for the biological samples.

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[*Received for review 23 September 1987; revised manuscript received 23 October 1987*]