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Cyclodextrin chiral stationary phases for liquid chromatographic separations of drug stereoisomers*

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Abstract: Many active drugs are racemic mixtures. Because the two enantiomers of a racemate often cause different pharmacological responses, the use of optically pure isomers is desirable and may be soon required. Cyclodextrin-bonded silica gel can be used as chiral stationary phase (CSP) in liquid chromatography. The enantiomers of 25 different racemic drugs were separated on such CSPs in the reversed-phase mode. The principal features of the cyclodextrin chiral recognition mechanism are recalled and some information on future trends for cyclodextrin CSPs is provided.

Keywords: *Chiral separation; cyclodextrin; liquid chromatography; racemic drugs.*

Introduction

One of the common threads of all living systems is that they are composed of the same types of chiral molecules. All naturally occurring amino-acids belong to the L-series. Racemic α-amino-acids have been detected in certain carbonaceous meteorites, indicating that those amino-acids were not produced by living forms. They were more likely produced by random processes. Also, most natural sugars are members of the D-series. Phospholipids, steroids, nucleotides, nucleosides, and bile salts are other examples of biological chiral materials.

It has been reported that about 57% of the active pharmaceutical compounds prescribed in the United States contain at least one chiral carbon atom [1, 2]. The biological activity of the two enantiomeric forms of an active drug can be significantly different. It was found that the L-form of thalidomide (*N*-(2,6-dioxo-3-piperidinyl)phthalimide) is a powerful teratogen and the D-form is a sedative and soporific drug [3, 4]. The administration of the racemate D,L-thalidomide to pregnant women was associated with a large number of newborn abnormalities. The drug was quickly withdrawn from this market. Another example of enantioselective biological activity is given by propranolol, the second most commonly prescribed drug in the United States. The (*S*) enantiomer of propranolol is about 100 times as potent as the (*R*) isomer [1, 5]. Such differences in pharmacological activity between enantiomers is often the rule rather than the exception. However, in the period 1983–1985, 88% of the new chiral drugs introduced were marketed as racemates [2]. It can be predicted that many racemic compounds will soon be considered as drugs with a 50% impurity [1, 2, 4].

Stereoisomeric separation is a very important analytical problem. Liquid chromatography (LC) and more recently gas chromatography (GC) are the most powerful techniques in enantiomeric separations [6, 7]. A chiral stationary phase (CSP) is most often used with a simple achiral mobile phase [6]. Among the available CSPs for LC are the polymeric chiral phases, the crown ether phases, the protein CSPs [8], the π-complex, hydrogen bond CSPs (Pirkle-type) [9], and the cyclodextrin (CD) bonded CSPs [6]. The latest CSPs were developed and introduced by Armstrong *et al.* in 1984 [10, 11]. The aim of this work is to present the separation of some drug stereoisomers using CD CSPs and hydroorganic mobile phases. The principal features of the CD chiral recognition mechanism are reiterated and some information on future trends for CD CSPs is provided.

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Experimental

Chemicals

Huperzine A, a drug under investigation for Alzheimer's dementia [12], was kindly provided by Professor A.P. Kozikowski and Dr Yan Xia of the University of Pittsburgh (PA). SQ 30840 is a drug under investigation at the Squibb Institute for Medical Research, Princeton (NJ). It was supplied by Dr Kimball. Efaroxan, Idazoxan and derivatives are drugs under investigation at Reckitt & Colman. They were supplied by N.A. Hyde (Danson Lane, Kingston-upon-Hull, UK). Fluoxetine is a drug under investigation at Eli Lilly (Indianapolis, IN). It was provided by Thomas L. Jeatran. Ibuprofen was supplied by Eli Lilly. The other drugs were obtained as described previously [13]. Etomidate and Norgestrel were supplied by Advanced Separation Technologies (Astec), Whippany (NJ). Methanol and acetonitrile were from Fisher Scientific (Fair Lawn, NJ).

Chromatography

The chromatographic system was a Shimadzu Gradient System, with two LC-6A pumps, a SCL-6A gradient controller, and a UV detector, model SPD-6A. The recorder was the Shimadzu model C-R2AX integrator. The column dimensions were 25 cm × 4.6 mm, i.d. Cyclobond I bonded with β-cyclodextrin (seven glucopyranoside units) from Astec. A Cyclobond II column (25 cm × 4.6 mm, i.d.) bonded with γ-cyclodextrin (eight glucopyranoside units) also was used.

Results and Discussion

Table 1 lists the names, formulae, and chromatographic conditions of the racemic drugs whose enantiomers were separated using CD-bonded CSPs. The k' values listed correspond to the first eluting enantiomer; α is the ratio of the first eluting k' value to the last eluting k' value, and R_s is the resolution factor.

All separated drugs, except norgestrel, contain at least one aromatic ring. As shown previously [11, 13, 14], the internal toroidal β-cyclodextrin (β-CD) cavity has a diameter varying from 0.6 nm at the bottom to 0.8 nm at the top of the cavity and is relatively non-polar. It can easily accommodate molecules or parts of molecules having the size of a five, six or seven-atom aromatic ring. The secondary 2- and 3-hydroxyl groups of each glucopyranoside ring stand axially at the mouth of the CD cavity, forming a polar crown that is most important for chiral recognition.

It was thought that there should be a difference in chiral CD recognition between compounds with the chiral centre as a part of a ring and those with the chiral centre in an acyclic portion of the molecule [15]. While this seems to be a factor, it also appears that the hybridization of the groups attached to the chiral centre have a significant effect. The highest resolution factor was obtained for compounds (i) whose chiral centre was part of a ring and (ii) whose chiral centre was linked to at least two sp^2 hybridized carbon atoms [15]. The highest resolution factor, $R_s = 3.7$, was obtained for compound SQ 30840 whose chiral centre is a part of a ring and is linked to three sp^2 hybridized carbon atoms. The lowest resolution factor, $R_s = 0.7$, was obtained for fluoxetine whose chiral centre is not part of a ring and is linked to only one sp^2 hybridized carbon atom.

The effects of substituents are demonstrated with the Idazoxan derivatives. Figure 1 shows the chromatogram of the methoxy, isopropenyl, and phenyl Idazoxan derivatives. The resolution was maximal with the two ether substituents (methoxy and ethoxy, $R_s = 2.5$) and with the isopropenyl substituent (sp^2 hybridized carbon atom, $R_s = 2.3$). The phenyl substituent, also contains a sp^2 hybridized carbon atom; however, it produced a slightly lower resolution factor ($R_s = 1.3$; Table 1). In this case, steric effects must be taken into account and it is likely that the bulky phenyl groups interfere somewhat with chiral recognition thereby negating the advantage of having an additional sp^2 hybridized carbon linked to the chiral centre.

The comparison of mephobarbital and mephenytoin is interesting: these two compounds are structurally identical, but the carbonyl group attached to the chiral centre of mephobarbital is missing in mephenytoin. Although the CD chiral recognition of the two compounds was similar ($R_s = 1.6$ and 1.8, $\alpha = 1.14$ and 1.33, respectively); the small carbonyl structural change made a tremendous difference in the global retention of the two molecules ($k' = 14.8$ and 0.48, respectively). It must be noted that part of the k' difference was due to the mobile phase composition (Table 1).

The size of the molecule is also an important

Table 1
Chromatographic parameters for the separation of enantiomeric drugs

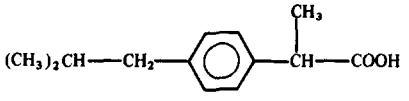
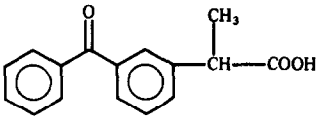
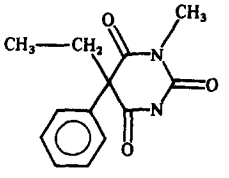
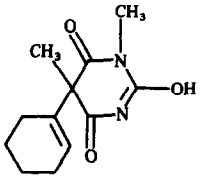
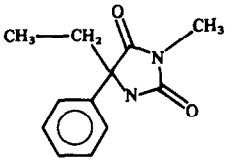
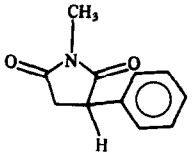
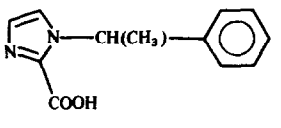
Therapeutic category and drug name	Formula	Mobile phase (%, v/v)	k'	α	R_s	Remarks
Anti-inflammatory agent						
Ibuprofen		MeOH–Buffer* 70:30	8.04	1.1	0.7	
Ketoprofen		MeOH–Buffer* 27:73	7.67	1.06	1.2	††,‡‡
Sedative, anticonvulsivant						
Hexobarbital		MeOH–Buffer* 15:85	9.39	1.14	1.5	††
Mephobarbital		MeOH–Buffer* 20:80	14.8	1.14	1.6	††
Mephenytoin		MeOH–Buffer* 40:60	0.48	1.33	1.8	††
Phensuximide		MeCN–Buffer* 10:90	1.97	1.15	1.5	††
Etomidate		MeCN–Buffer† 10:90	2.7	1.46	0.9	
		MeCN–Water‡ 5:95	1.8	1.70	1.1	

Table 1
Continued

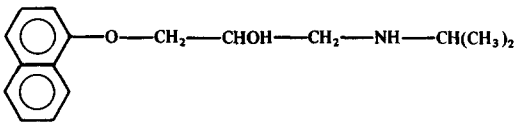
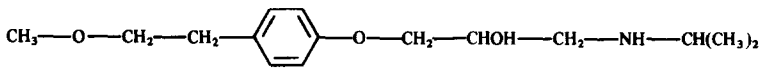
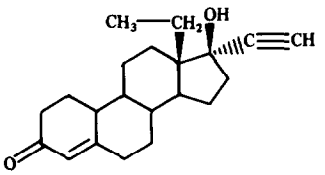
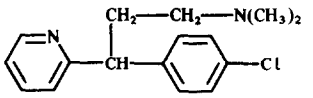
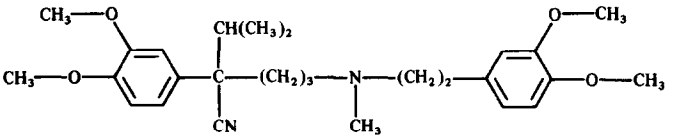
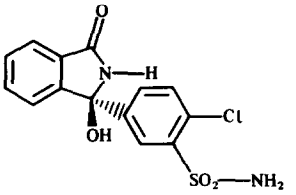
Therapeutic category and drug name	Formula	Mobile phase (%, v/v)	k'	α	R_s	Remarks
β-adrenergic blocker						
Propranolol		MeOH–Buffer* 25:75	2.78	1.04	1.4	††,‡‡
Metoprolol		MeOH–Buffer* 32:68	3.51	1.03	0.9	††,‡‡
Progestrin						
Norgestrel		MeCN–Water 30:70	0.48	1.24	1.1	§§
Antihistamine						
Chlorpheniramine		MeCN–Buffer* 15:85	5.86	1.07	1.5	††
Vasodilator						
Verapamil		MeCN–Buffer* 15:85	2.94	1.03	0.7	††,§§
Diuretic						
Chlorthalidone		MeOH–Buffer* 30:70	0.50	1.44	1.9	††

Table 1
Continued

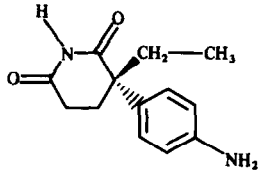
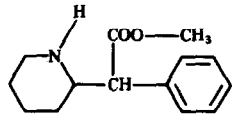
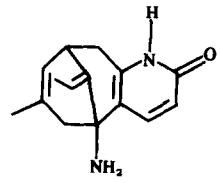
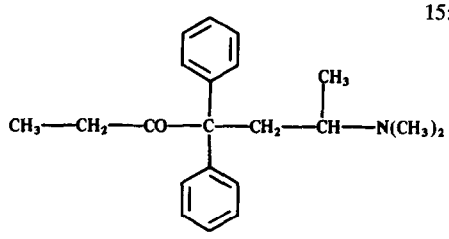
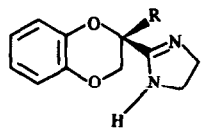
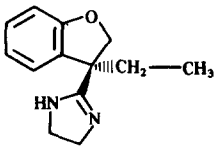
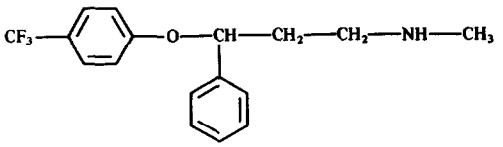
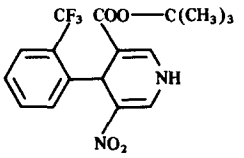
Therapeutic category and drug name	Formula	Mobile phase (%, v/v)	k'	α	R_s	Remarks
Anticorticosteroid						
Aminoglutethimide		MeCN–Buffer* 15:85	7.49	1.03	0.9	††,‖‖
Central nervous system stimulant						
Methylphenidate		MeCN–Buffer* 10:90	1.17	1.14	1.6	††,‡‡
Huperzine A		MeCN–Buffer** 10:90	1.83	1.05	0.8	
Narcotic, analgesic						
Methadone		MeCN–Buffer* 15:85	2.38	1.04	0.8	††,‖‖
Compounds under pharmacological investigation						
Idazoxan derivatives						
R = ethyl		Buffer§	5.74	1.19	1.3	
R = isopropenyl		MeOH–Buffer‖ 10:90	3.20	1.30	2.3	
R = phenyl		MeOH–Buffer‖ 10:90	6.12	1.19	1.3	
R = methoxy		MeOH–Buffer‖ 10:90	1.21	1.40	2.5	
R = ethoxy		MeOH–Buffer* 10:90	1.62	1.40	2.5	

Table 1
Continued

Therapeutic category and drug name	Formula	Mobile phase (%, v/v)	k'	α	R_s	Remarks
Efaroxan		MeOH–Buffer* 5:95	2.55	1.2	1.1	
Fluoxetine		MeOH–Buffer* 40:60	5.02	1.1	0.7	
SQ 30840		MeCN–Buffer* 20:80	2.33	1.39	3.7	

Column 25 cm × 4.6 mm, i.d. Cyclobond I (β-CD). Mobile phase composition indicated, MeOH = methanol, MeCN = acetonitrile, flow rate 1 ml min^{-1}. Room temperature.
*1% (w/v) Triethylammonium acetate in water (TEAA), pH 4.1.
†5% (w/v) Triethylammonium acetate in water (TEAA), pH 7.
‡0.01% (w/v) Diethylamine in water.
§1% (w/v) Tricthylammonium acetate in water (TEAA), pH 5.98.
‖1% (w/v) Triethylammonium acetate in water (TEAA), pH 4.2.
**1% (w/v) Triethylammonium acetate in water (TEAA), pH 7.
††Adapted from ref. 13.
‡‡Two 25-cm Cyclobond I columns were used in series.
§§A Cyclobond II column was used (γ-CD).
‖‖A gradient from 10% MeCN to 20% MeCN in 20 min was used; mean composition MeCN 15% (v/v).

parameter in chiral recognition by CD. If the molecule is too big to fit inside the CD cavity, any chiral recognition is hindered. This was the case for norgestrel. The two isomers of this steroid were not separated by the β-CD-bonded column. The chiral centre of norgestrel is part of a ring and has an *sp* hybridized carbon atom. Although there is no aromatic ring in this molecule, it should be possible to obtain a chiral separation on CD-bonded CSPs. Figure 2 shows the separation obtained on a γ-CD-bonded column. γ-CD has eight glucose units and a larger cavity that can accommodate steroids.

Figure 3 is the circular dichroism spectrum of the two peaks obtained with huperzine A. Huperzine A was injected five times and its first and second peaks were collected in two different vials. The circular dichroism of each collected peak was obtained using a Jasco J600 polarimeter. The two circular dichroism spectra were superimposed in Fig. 3. For any wavelength, the circular dichroism of the first peak is opposite to the one of the second peak. This is absolute proof of enantiomeric separations. However, this experiment did not allow the determination of the absolute chemical configuration of the enantiomers. Indeed, the

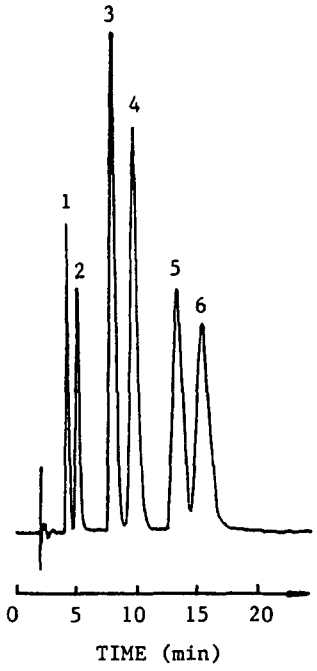


Figure 1
Separation of Idazoxan derivatives. Column: β-CD 250 × 4.6 mm, i.d. Mobile phase: 95% buffer (1% TEAA, w/v; pH = 4.2)–5% acetonitrile (v/v), 1.5 ml min^{-1}. Detection: UV 270 nm, 0.01 AUFS. 1, 2, Methoxy derivatives; 3, 4, isopropenyl derivatives; 5, 6, phenyl derivatives.

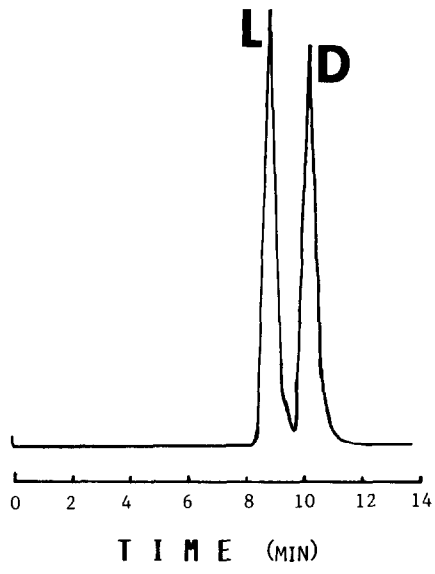


Figure 2
Separation of D- and L-Norgestrel on a 25-cm γ-CD column. Mobile phase: acetonitrile–buffer (TEAA 1%, w/v) 30:70, v/v, 1 ml min^{-1}.

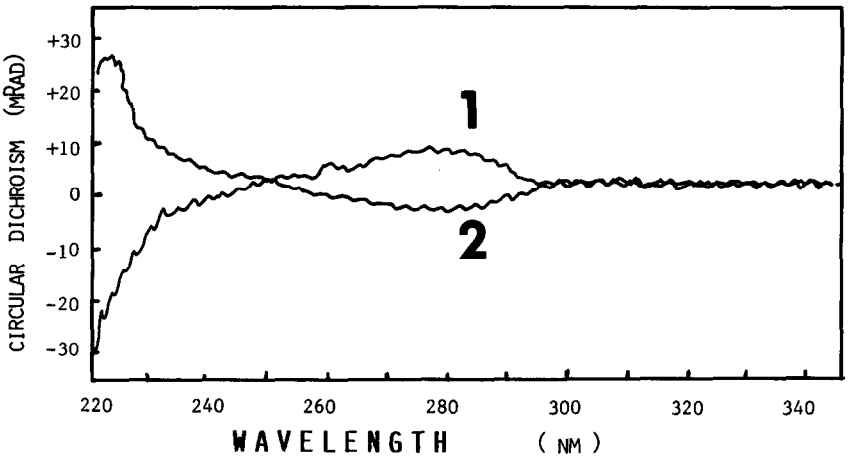


Figure 3
Circular dichroism superimposed spectra of peaks 1 and 2 obtained with huperzine A.

D and L structures are not systematically linked to a positive or negative light polarization rotation.

Triethylammonium acetate (TEAA) was added at relatively high concentration (0.062 M or 1% w/v) in the aqueous fraction of the mobile phases to decrease the retention and to improve the efficiency of amino compounds. Basic amino groups have a high affinity for the hydroxyls on the CDS. This may produce high retention times and peak tailing. The addition of 1%, w/v, of TEAA to the mobile phase can solve this problem; the TEA^+ ions bind to the silanol groups thereby

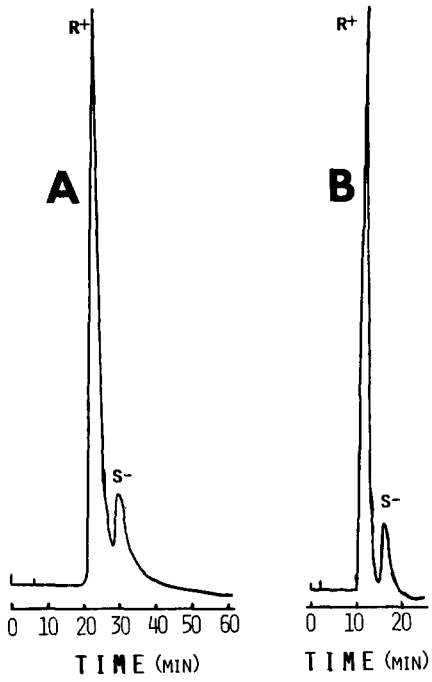


Figure 4
Separation of (*R*) and (*S*) etomidate. A, two 25-cm Cyclobond I columns connected in series; mobile phase, buffer (1% TEAA, pH 7)–acetonitrile (90:10, v/v), 0.9 ml min^{-1}. B, one 25-cm Cyclobond I column; mobile phase, water (0.01%, v/v diethylamine, pH 9.5)–acetonitrile (95:5, v/v), 0.8 ml min^{-1}.

producing sharper peaks for amino compounds [13]. Figure 4 shows the evolution of the separation of the etomidate enantiomers. The use of a diethylamine (DEA, 0.01% v/v) solution (95% and 5% acetonitrile, v/v) produced an even better separation. With two columns in series using the TEAA buffer, the separation and resolution factors were 1.46 and 0.9, respectively (Fig. 4A). With only one column and the DEA mobile phase, the separation and the resolution factors were 1.70 and 1.3, respectively (Fig. 4B). It must be noted that the pH of the DEA mobile phase was 9.5. This high pH value can damage the column irreversibly by dissolving the silica, cleaving some Si—C bonds and releasing the bonded CDs. However, for short periods of time (as in this separation), it is sometimes advantageous to use mobile phases at higher pH values.

The potential of CSPs based on CDs is tremendous and recently CD derivatives that can be used as CSPs in gas chromatography have been developed ([16]; and Berthod, Jin and Armstrong, to be published). Derivatized CDs also can be used in LC to improve chiral separations or to extend the applicability of such CSPs. Currently this group is completing experiments on the use of derivatized CDs as stationary phases for both reversed and normal-phase LC. These phases are able to resolve enantiomers that cannot be separated by the traditional native CD stationary phases. As recently reviewed by Wainer [17], the use of CSPs in LC is a new technology that can improve dramatically the quality of chiral drugs. It can be predicted that a full clinical study of the pharmacological and toxicological effects of both enantiomers of any new chiral drug may be required soon to obtain an authorization for marketing the drug as a racemic mixture [2].

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