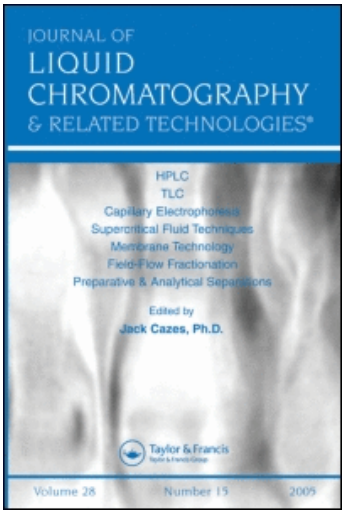

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Enantiomeric HPLC Separation of Selected Chiral Drugs Using Native and Derivatized β-Cyclodextrins as Chiral Mobile Phase Additives

Emmanuel Ameyibor[a]; James T. Stewart[a]
[a] Dept. of Medicinal Chemistry, College of Pharmacy The University of Georgia, Athens, GA

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ENANTIOMERIC HPLC SEPARATION OF SELECTED CHIRAL DRUGS USING NATIVE AND DERIVATIZED β-CYCLODEXTRINS AS CHIRAL MOBILE PHASE ADDITIVES

Emmanuel Ameyibor, James T. Stewart

Dept. of Medicinal Chemistry
College of Pharmacy
The University of Georgia
Athens, GA 30602-2352

ABSTRACT

Native β-Cyclodextrin and three of its derivatives, hydroxypropyl, methyl and sulfated β-cyclodextrins were used as chiral mobile phase additives to investigate the enantiomeric separation of selected drugs by reversed phase HPLC. The chiral drugs investigated were oxazepam, temazepam, lorazepam, ketoprofen, fenoprofen, ibuprofen, chlorthalidone, terbutaline, trimeprazine and trimipramine. Different types of reversed phase columns were investigated including propylsilane, hexylsilane, octylsilane, octadecylsilane columns and a new nonporous octadecylsilane column. The new nonporous column gave separations for most of the drugs including short retention times. Terbutaline was not separated on the nonporous column, but did separate on hexylsilane and octylsilane columns. The effect of various mobile phases, organic modifier, type and concentration of the added cyclodextrin on peak shape, resolution and retention factors of the enantiomers were investigated.

INTRODUCTION

When a chiral selector is introduced into a mobile phase used with an achiral column, it offers advantages of flexibility, a wide range of possible additives, and often lower cost compared with an equivalent chiral stationary phase, in addition to avoiding the disadvantages associated with indirect chiral methods. Cyclodextrins (CDs) are widely used as chiral mobile phase additives.[1-6] They are cyclic, non-reducing oligosaccharides consisting of D-glucose units bonded through alpha- 1,4-linkages. According to the number of glucose units forming the cyclodextrin ring (six, seven or eight), one differentiates between alpha, beta and gamma cyclodextrin. The most commonly used CD is the β-CD. The outer surface of the CD molecule is hydrophilic due to the presence of primary and secondary hydroxyl groups, whereas the inner cavity is hydrophobic.[7]

When CDs are used as chiral mobile phase additives in reversed phase liquid chromatography, the separation mechanism is thought to be the result of formation of inclusion complexes in which the solute is included in the cavity of the CD. For enantiomers, chiral recognition occurs because of the selective complexation of the enantiomers with the CDs. Among the factors that control the enantioseparation process are, (i) differences in the stability/binding constants of the CD complexes, (ii) differences in the adsorption of CD complexes on the surface of the stationary phase and (iii) differences in the adsorption of free solute molecules on the CD layer that is adsorbed on the surface.[8] Because the enantioselective complexation is enhanced with stabilized inclusion of the hydrophobic part of the solute in the cavity of the CDs, the cavity size of CD is of great importance in inclusion complex formation. Only those guest molecules which can be fitted into the chiral cavity of the CD, resulting in intimate contact with the inner surface, can form stable inclusion complexes. If the size of the molecule is too small or too large, either a weak or no interaction is formed leading to little or no separation.

Furthermore, to obtain a chiral separation with CD, it is necessary for different interactions to take place between the enantiomers and the CD. These interactions include, dipole-dipole interactions, inductive, hydrogen bonding and hydrophobic (Van der Waals) interactions. If at least one of these interactions is stereochemically dependent, chiral separation of an enantiomeric solute is possible.[9]

Problems associated with the use of CDs are due to their poor solubility in aqueous organic solvents. The addition of urea to the mobile phase will enhance solubility, but it sometimes leads to problems with baseline stability

and a higher viscosity of the mobile phase.[10] An alternate way is to chemically modify one of the secondary hydroxyl groups of the CD. Chemical modification changes both the chiral selectivities and physical properties of the CD such as the strength and the nature of the polar intermolecular interactions between the host and the guest and the solubility of the materials.[11]

The differences in inclusion complex strengths between solutes and the CD cavity, as well as differences in the interaction with the rim functional groups can result in improved chromatographic separations. For chemically modified CDs, the hydroxyl groups on the rim of the cavity are replaced with either methyl, hydroxyethyl, hydroxypropyl, sulfate and acetyl groups to increase the hydrophobic character of the CD cavity relative to the hydrophilic exterior. These differences will change the inclusive complex strength which can lead to greater selectivity.[12]

In this study, the chiral chromatographic behavior of propylsilane, hexylsilane, octylsilane, octadecylsilane columns and a new nonporous octadecylsilane column were investigated with native and derivatized βCDs added to the mobile phase. The derivatized CDs were hydroxypropyl-β-CD, methyl-β-CD and sulfated-β-CDs. The chiral drugs investigated were chlorthalidone, terbutaline, oxazepam, lorazepam, temazepam, ketoprofen, ibuprofen, fenoprofen, trimeprazine and trimipramine. The most attractive features of the nonporous column are the use of low quantities of organic modifier in the mobile phase and very fast retention times.

EXPERIMENTAL

Materials

β-Cyclodextrin (β-CD) was purchased from TCI (Portland, Oregon, USA), hydroxypropyl β-Cyclodextrin (HP-β-CD-degree of substitution 4.0), methyl β-Cyclodextrin (M-β-CD-degree of substitution 12.7) and sulfated β-Cyclodextrins (S-β-CD-degree of substitution 14) were kindly supplied by American Maize Company (Hammond, IN, USA). Terbutaline hemisulfate, lorazepam, temazepam, ketoprofen, ibuprofen, trimeprazine hemi-(+)-tartrate and trimipramine maleate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Chlorthalidone and fenoprofen reference standards were obtained from USP (Rockville, MD, USA). Acetonitrile and absolute methanol were purchased from J. T. Baker (Phillipburg, NJ, USA).

Triethylamine (TEA) was obtained from Fisher Scientific Co. (Orangeburg, NY USA) and trifluoroacetic acid (TFA) was purchased from Aldrich Chemical Co (Milwaukee, WI, USA). All solvents were HPLC grade and mobile phases were filtered through a 0.45μm filter (Alltech Associates, Deerfield, IL. USA).

Instrumentation

Chromatography was performed on an HPLC system consisting of a Beckman Model 110A solvent delivery module (Beckman, San Ramon, CA, USA), a Rheodyne 7125 injector (Rheodyne, Cotati, CA, USA) equipped with a 10μL loop and a Waters Millipore Model 481 LC spectrophotometer (Milford, MA 01757, USA). A Spectra-Physics Model 4270 integrator (Spectra-Physics, San Jose, CA, USA) was used to record each chromatogram and peak area responses.

The following HPLC columns were purchased: Zorbax 300 SB-C3 (Mac-Mod Analytical Inc., Chadds Ford, PA, USA), Spherisorb 5C6 (150x4.6mm), Spherisorb S5C8 (150x4.6mm), Spherisorb S50DS1 (250x4.6mm, Phenomenex, Torrance, CA, USA) and a Micra Nonporous reversed phase (RP) C18 1.5μ HPLC column (33x4.6mm, Micra Scientific, Northbrook, IL, USA).

Chromatographic Conditions

All separations were performed at ambient temperature (23°C) with UV detection set at the UV maximum for each analyte. The maxima were 240nm for oxazepam, lorazepam and temazepam, 265nm for ketoprofen, fenoprofen and ibuprofen, 275nm for terbutaline and chlorthalidone and 250nm for trimipramine and trimeprazine.

The mobile phases consisted of 5 to 20mM concentrations of native or derivatized CDs dissolved in 0-10% v/v acetonitrile and 90-100% v/v of 0.1% aqueous trifluoroacetic acid (pH adjusted to 4.0 with triethylamine). The flow rate was 0.8mL/min. for the nonporous column and 1.0mVmin. for the other columns. Stock solutions of 1μg/mL of each individual analyte were prepared in the respective mobile phases.

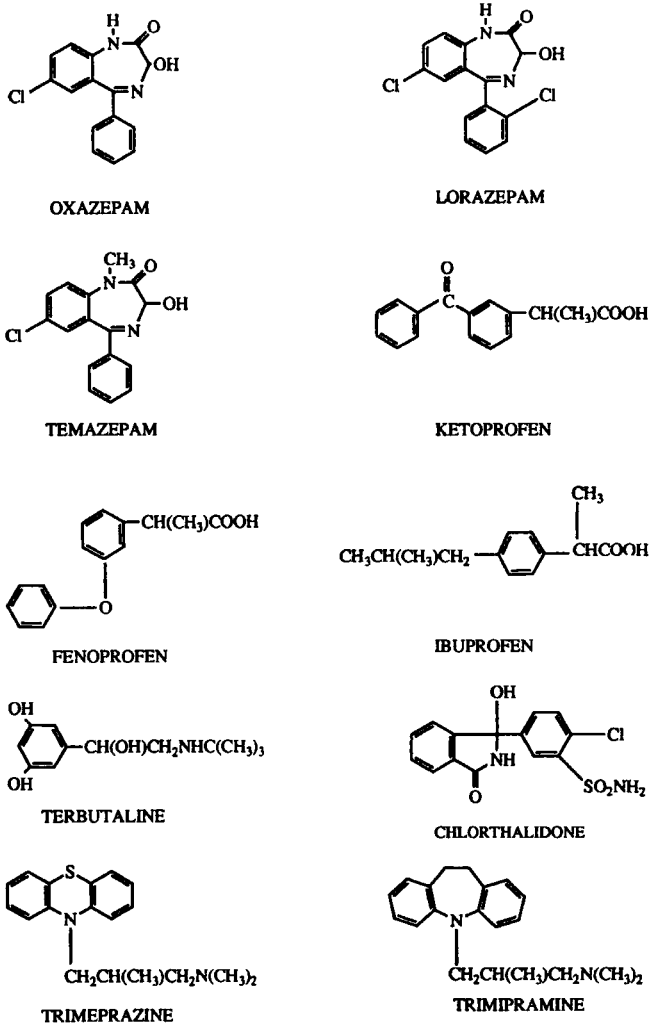

Figure 1. Chemical structures of analytes studied.

RESULTS AND DISCUSSION

The chemical structures of the analytes investigated are shown in Fig.1. Preliminary results in this laboratory showed that only chlorthalidone and terbutaline enantiomers were resolved on the hexylsilane and octylsilane columns with either β-CD or HP-β-CD added to the mobile phase. Mobile

phase conditions for chlorthalidone were 10mM β-CD or HP-β-CD dissolved in water:methanol (9: 1 v'v) pH4.0. The terbutaline enantiomers did not require an organic modifier for resolution and there was a complete loss of resolution upon the addition of any organic modifier to the mobile phase. The use of a 50mM ammonium acetate buffer gave sharper peaks for both chlorthalidone and terbutaline enantiomers on the hexylsilane and octylsilane columns. None of the analyses investigated in this study including chlorthalidone and terbutaline gave separations on the classical octadecylsilane or the stable bond propylsilane columns with β-CD, HP-β-CD, M-β-CD or S-β-CD added to the mobile phase.

The recently introduced nonporous reversed phase octadecylsilane column which had shown initial success for separation of chlorthalidone enantiomers was studied. Separations were achieved for oxazepam, temazepam, lorazepam, fenoprofen, ketoprofen, trimipramine, trimeprazine and chlorthalidone. Terbutaline and ibuprofen enantiomers could not be separated. Only trimipramine and trimeprazine were separated with native β-CD and ketoprofen and fenoprofen separated only with HP-β-CD added to the mobile phase. Oxazepam, temazopam and lorazepam separated with either HP-β-CD or M-β-CD and chlorthalidone was the only analyte where the enantiomers were separated with either β-CD or HP-β-CD. S-β-CD failed to resolve any of the analytes. In cases where enantioseparation was achieved, the mobile phase consisted of CDs added to solutions of 0.1% aqueous trifluoroacetic acid and acetonitrile and the presence of buffers in the mobile phase did not improve the separations.

Based on the initial success of the nonporous column, the chromatographic behavior of this column was further investigated. Tables 1 and 2 list the various mobile phase compositions and the retention factors of the analytes. In almost all cases, the resolution between enantiomeric pairs was greater than 1. In addition to the nonporous nature of the stationary phase, other unique features of the column are the requirement of a low concentration of organic modifier and the short retention times for analysis as shown in Figs 2 and 3.

Effect Of CD Type on Enantiomeric Separation

Chemical modification of the CD has been shown to 'stretch' the cavity mouth and therefore change the hydrophobicity of the molecules and the stereoselectivity of the inclusion process. The mouth of the CD hydrophobic cavity is surrounded by secondary hydroxyl groups which are locked into

Table 1

Resolution (Rs) of Lorazepam, Temazepam, Oxazepam, and Chlorthalidone Enantiomers on Nonporous Octadecylsilane Column

Analyte	Mobile Phase Composition (v/v)[a]		Retention Factors		
	A	B	k_1	k_2	Rs
Lorazepam	98	2	6.04	7.00	1.05
	96	4	6.60	7.56	1.05
	94	6	6.25	7.37	1.02
	92	8	6.54	7.33	1.00
	90	10	6.46	7.13	0.90
Temazepam	98	2	8.58	10.85	1.34
	96	4	8.25	10.10	1.25
	94	6	7.23	8.54	1.14
	92	8	7.42	8.50	1.10
	90	10	7.37	8.22	1.00
Oxazepam	98	2	3.79	5.60	1.97
	96	4	4.66	6.51	1.90
	94	6	4.19	6.00	1.74
	92	8	4.60	6.16	1.74
	90	10	4.11	5.50	1.65
Chlorthalidone	98	2	4.27	5.79	1.78
	96	4	--[b]	--	--
	94	6	--	--	--
	92	8	--	--	--
	90	10	--	--	--

[a] aqueous 0.1% TFA pH 4.0 (adjusted with TEA) - acetonitrile containing 15mM HP-β-CD. A = aqueous 0.1% TFA pH 4.0 (adjusted with TEA). B = Acetonitrile. [b] No resolution was achieved.

position and are considered to be important in chiral recognition.[13] In a derivatized CD, some hydroxyl groups are substituted with various functional groups, such as hydroxypropyl, methyl, sulfate and acetyl. The overall hydrophobic character of the CD will depend on the type of functional

Table 2

Resolution (Rs) of Trimeprazine, Timipramine, Ketoprofen, and Fenoprofen Enantiomers on Nonporous Octadecylsilane Column

Analyte	Mobile Phase Composition (v/v)[a]		Retention Factors		
	A	B	k_1	k_2	Rs
Trimeprazine	98	2	8.14	10.60	1.25
	96	4	7.90	10.44	1.10
	94	6	7.74	9.96	1.00
	92	8	--[b]	--	--
	90	10	--	--	--
Trimipramine	98	2	3.43	4.42	1.33
	96	4	4.53	7.26	1.10
	94	6	3.52	4.42	1.03
	92	8	--	--	--
	90	10	--	--	--
Ketoprofen	98	2	4.10	5.21	1.25
	96	4	4.15	5.06	1.00
	94	6	--	--	--
	92	8	--	--	--
	90	10	--	--	--
Fenoprofen	98	2	6.41	7.48	1.15
	96	4	6.19	7.19	1.00
	94	6	--	--	--
	92	8	--	--	--
	90	10	--	--	--

[a] aqueous 0.1% TFA pH 4.0 (adjusted with TEA) - acetonitrile containing 10mMβ-CD for trimiprazine and trimipramine and 15mM HP-β-CD for ketroprofen and fenoprofen. A = aqueous 0.1% TFA pH 4.00 (adjusted with TEA). B = acetonitrile. [b] No resolution was achieved.

groups present. In addition to the inclusion complex formed with a derivatized CD, modification of the CD rim is also critical for the type of interaction between the functional groups on the rim and the portion of the analyte outside the cavity that must take place for chiral recognition to occur. Included are

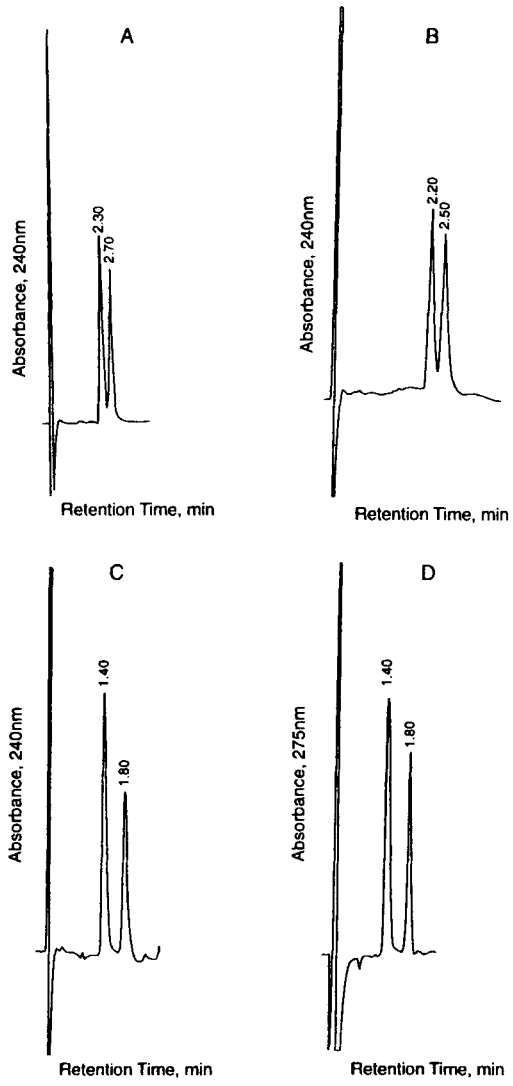

Figure 2. Enantiomeric separations of (A) lorazepam, (B) temazepam, (C) oxazepam and (D) chlorthalidone on the nonporous octadecylsilane column. Mobile phase consisted of 98:2 v/v aqueous 0.1% TFA pH 4.0 (adjusted with TEA) - acetonitrile containing 15mM HP-β-CD at a flow rate of 0.8mL/min.

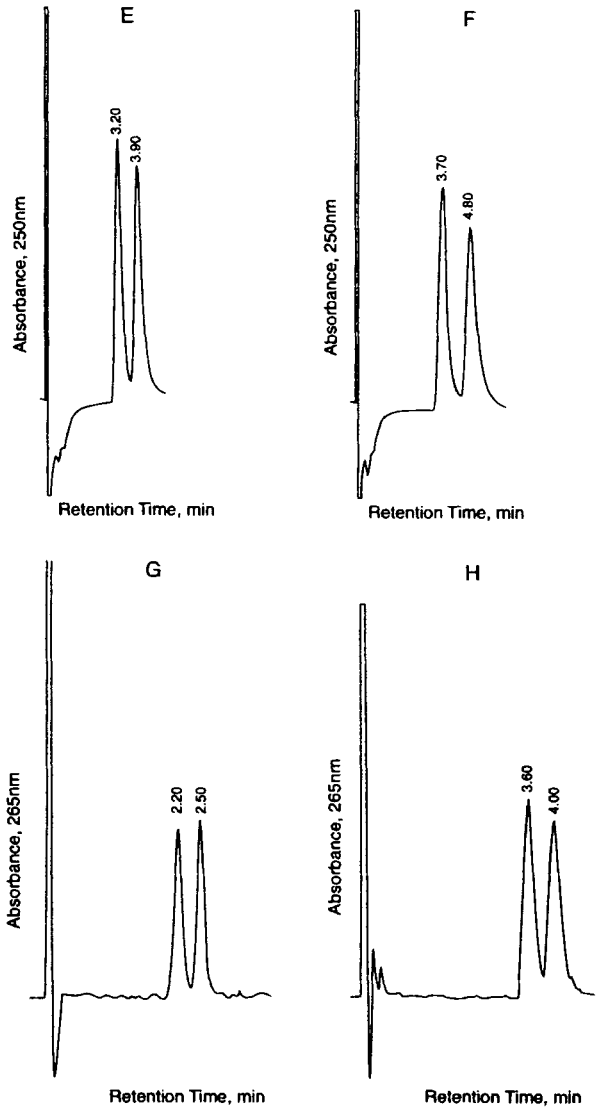

Figure 3. Enantiomeric separations of (E) trimeprazine, (F) trimipramine, (G) ketoprofen and (H) fenoprofen on nonporous octadecylsilane column. Mobile phase consisted of 98:2 v/v aqueous 0.1% TFA pH 4.0 (adjusted with TEA) - acetonitrile containing 10mM β-CD (for E and F) and 15mM HP-β-CD (for G and H) at a flow rate of 0.8mL/min.

hydrophobic (Van der Waals), hydrogen bonding and dipole-dipole interactions.[14] In contrast to the secondary hydroxyl groups which are locked into position on the native CD, the hydroxyl moiety of the hydroxypropyl group is free to rotate. This flexibility may allow for a closer approach between the hydroxyl groups and any hydrogen bonding moiety present in the solute leading to stronger or more stereospecific interactions than are possible with the native CD.

In the case of M-β-CD, Van der Waals interactions between the methyl groups on the CD rim and the hydrophobic groups on the solute chiral center can also provide enantioselectivity.[14]

Oxazepam, temazepam and lorazepam enantiomers were resolved with HP-β-CD but not with β-CD. The resolution occured because, in addition to the aromatic ring structures for inclusion in the cavity of the CD, all three analytes have a hydroxyl group at the chiral center which is available for specific hydrogen bonding interactions with the rim hydroxypropyl groups of HP-β-CD.

The same phenomenon explains the results we obtained for fenoprofen and ketoprofen. In addition to the two aromatic rings which form inclusion complexes with CD, both have carboxylic acid groups at the chiral center taking part in additional interactions with the functional groups on the rims of the derivatized CDs. Ibuprofen has only one aromatic ring compared to the other profens and is only partially resolved because of the small size of the molecule and hence its inability to form a strong or tight inclusion complex with the CD cavity.

It is interesting to note that trimipramine and trimeprazine enantiomers which failed to separate with HP-β-CD do not have any hydrogen bonding functional groups at or near the chiral center to interact with the hydroxypropyl or methyl groups on the rim of the CD.

The bulky nature of both analyses form strong inclusion complexes with native β-CD and the side chains interact favorably with the CD rim. Chlorthalidone was the only analyte to separate with either β-CD or HP-β-CD. This is because the bulky groups of chlorthalidone form an inclusion complex with β-CD and also the carbonyl and hydroxyl functional groups are available for hydrogen bonding interactions with the rim hydroxypropyl groups of the HP-β-CD.

Effect of Organic Modifier Concentration

One of the unique characteristics of the nonporous reversed phase column was the relatively low organic modifier concentration needed for chromatographic analysis. Compared to classic reversed phase columns, only about one-third of the organic modifier concentration used for a typical analysis is required. This is even more important in chiral mobile phase additive separations because the addition of organic modifier to the mobile phase is known to greatly decrease the solubility of CDs.[7] When CDs form complexes with analytes, it is assumed that the hydrophobic portion of the analyte sits inside the hydrophobic cavity of the CD and therefore the addition of organic modifier reduces the affinity of the analyte for the CD. The organic solvent competes with the solute for the preferred locations in the hydrophobic cavity resulting in various degrees of interactions of the compounds with the CD. Increasing the organic content of the mobile phase will weaken the strength of the inclusion complex formed between the analyte and the CD.[15]

The retention profiles of the analytes studied followed the general model where the capacity factor decreases with increasing organic modifier concentration, as in a classic reversed phase system consisting of an octadecylsilane stationary phase and a buffered aqueous-organic eluent.[16] In general, resolution decreased with increasing acetonitrile concentration for all the analyses in this study though the effect was less pronounced for temazepam, lorazepam and oxazepam.

For fenoprofen, ketoprofen, trimeprazine and trimipramine, enantioselectivity was lost when the acetonitrile concentration in the mobile phase was more than 6%. For chlorthalidone, enantioselectivity was lost when the acetonitrile concentration was only 2%, indicating an interference of the organic modifier with complexation of the analytes and CDs. Other organic modifiers such as methanol and ethanol were also investigated, but acetonitrile gave the best results.

Effect of Native β-CD and HP-β-CD Concentration

Table 3 lists the effect of β-CD or HP-β-CD concentrations in the mobile phase on the enantiomeric separation of the analytes. Enantioselectivity generally improved with increasing β-CD or HP-β-CD concentration in the mobile phase. It is thought that each enantiomer forms an inclusion complex upon the addition of at least 5mM of CD to the mobile phase and the two inclusion complexes have different capacity factors.

Table 3

Effect of CD Concentration on Resolution of Lorazepam, Temazepam, Oxazepam, Chlorthalidone, Trimeprazine, Trimipramine, Ketoprofen and Fenoprofen Enantiomers on Nonporous Octadecylsilane Column

Analyte	CD Concentration (mM)							
	5		10		15		20	
	HP-β-CD	β-CD	HP-β-CD	β-CD	HP-β-CD	β-CD	HP-β-CD	β-CD
Lorazepam	0.78	--[a]	1.05	--	1.05	--	1.00	--
Temazepam	1.00	--	1.08	--	1.89	--	1.22	--
Oxazepam	1.00	--	1.30	--	1.97	--	1.80	--
Chlorthalidone	1.10	1.00	1.35	1.85	2.50	1.20	1.72	0.90
Trimeprazine	--	0.84	--	1.25	--	1.10	--	0.93
Trimipramine	--	0.95	--	1.34	--	1.24	--	1.12
Ketoprofen	0.83	--	1.13	--	1.30	--	1.10	--
Fenoprofen	0.74	--	0.90	--	1.20	--	1.05	--

Mobile phase consisted of 98:2v/v aqueous 0.1% TFA pH 4.0 (adjusted with TEA) - acetonitrile containing the appropriate concentration of CD at a flow rate of 0.8 mL/min.
[a] No resolution was achieved.

When the concentration of CD falls below 5mM, the formation of the inclusion complex is incomplete and there is either partial or no resolution of the enantiomers.[17] Consequently, the concentration of CD is very important for resolution.

Increasing the β-CD or HP-β-CD concentrations in the mobile phase resulted in an increase in resolution of the enantiomers indicating the formation of relatively strong inclusion complexes. However, the increase in resolution reached a plateau region for all the analyses after which there was decreased resolution when the CD concentration was increased. This may be due to the increased bulk of the hydroxypropyl groups which would be expected to lead to a reduction of interactions with the secondary hydroxyl groups on the rim of the CD and result in reduction of enantiomeric resolution.

In conclusion, both the hexylsilane and octylsilane columns were successful in the enantiomeric separations of chlorthalidone and terbutaline among the analytes studied. The nonporous reversed phase column was shown to be applicable to enantiomeric separations of a group of diverse chiral drugs. In addition to short retention times, the use of low organic modifier concentration in the mobile phase offers a wide range of chromatographic applications for the column. In general, hydroxypropyl-β-CD as a chiral

mobile phase additive offers a wide variety of advantages over β-CD in terms of the ease of formation of inclusion complexes and the additional number of interactions possible with various functional groups present in drug molecules.

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