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Resolution of promethazine, ethopropazine, trimeprazine and trimipramine enantiomers on selected chiral stationary phases using high-performance liquid chromatography

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Abstract

The separation of the enantiomers of three phenothiazines and a dibenzazepine were investigated on several different chiral stationary phases without the use of derivatization or column switching. The phenothiazines selected were promethazine, ethopropazine and trimeprazine and the dibenzazepine was trimipramine. Three classes of columns were studied for their ability to separate the enantiomers of these compounds: brush or Pirkle, inclusionary, and affinity types. The columns studied were (α-*R*-naphthyl)ethylurea, (*S*)-*tert.*-leucine-(*R*)-1-(α-naphthyl) ethylamine, dinitrobenzoylphenylglycine; β-cyclodextrin, β-acetylated cyclodextrin; γ-cyclodextrin; Chiralcel-OD; Chiralcel-OJ and Ovomucoid. Changes in mobile phase composition were studied on each column to determine the effects of solvents on the separation of the various enantiomers. Simplex calculations for mobile phase variations were not performed because they do not predict elution order reversals.

1. Introduction

Interest in this laboratory in the separation of enantiomers of phenothiazines and dibenzazepines led to an investigation of mobile phase and stationary phase interactions and their influences on enantiomeric resolution. Among the drugs of interest were promethazine, ethopropazine, trimeprazine and trimipramine. Some success has been reported for promethazine and trimipramine on the α_1-acid glycoprotein (α_1-AGP) protein column [1–4]. Other separations for promethazine, trimeprazine and trimipramine enantiomers have been performed either by chemical derivatization of the compounds, or using cyclodextrins (CDs) as mobile phase additives [1–5].

Chiral stationary phases (CSPs) are categorized under several different types of chromatography including brush type, inclusionary and affinity. A brush-type CSP is also called a Pirkle CSP. It relies upon multiple discreet molecular interactions to discriminate between enantiomers. These interactions are dipole–dipole, π–π, steric hindrance and H bonding [6–9]. Pirkle-type columns have a wide range of physical compositions ranging from amino acids to substituted naphthyl rings and practically every com-

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mercial chromatographic supplier offers a chiral column of this type. A survey of the literature shows that Pirkle-type columns were the most successful type of CSP in the early 1980s [7–11].

CDs compose a major portion of the inclusionary type of columns. They have a toroidal geometry with a hydrophilic outer surface and a hydrophobic inner cavity. The three most common CDs are α, β and γ which are made up of 6, 7 and 8 α-D-glucopyranose units, respectively. In the reversed-phase mode, CDs separate enantiomers by a difference in energies between the two diastereomeric inclusionary complexes [8,9]. A study of the literature showed that the separation of a wide range of chiral isomers should be possible using silica-bound CD columns in the reversed-phase mode [8,9].

Other inclusionary-type CSP such as the cellulose-based CSP also utilize diastereomeric complexing energy differences to obtain separations. The Chiralcel CSPs are the most successful type of CSP since 1986 [8–12]. The free hydroxyl groups on cellulose provide an environment conducive to derivatization to form esters and carbamates; thus, allowing them to be chemically varied as much as CDs. But the cellulose-based CSP may have channels rather than the cavities of the CDs. The channels have size, capacity and structural limitations somewhat similar to those of the CDs. There is some question as to the exact nature of the cavities associated with the cellulose stationary phases. Some indicate that the cellulose material is not necessarily a channel, but an intricate crosswork of cellulose strands forming asymmetric cavities in which the chiral resolution occurs. Evidence exists in literature which argues both pro and con for these hypotheses. Analytes for which enantiomeric separations may be successful, should be aromatic, have H-bonding capacity, and not too large a structure to fit in the tubular chiral cavity. The Chiralcel-OD stationary phase, a carbamate derivative of cellulose, has been used to separate a wide variety of chiral compounds including chiral alcohols and chiral amines. The Chiralcel-OJ column is a benzoyl ester derivative of cellulose and has been used to separate compounds requiring additional $\pi-\pi$ interactions. The cellulose-based columns differ from CD columns in that they have severe mobile phase limitations [8,9].

Affinity chromatography utilizes the ionic formation of the amino acids in proteins to provide stereoselective regions of H bonding, hydrophobic conditions and electrostatic forces that have differing interactions between the (*R*) and (*S*) enantiomers of guest molecules [8,9]. Protein-type CSPs are versatile in their ability to work with both cationic and anionic drugs. In the past, protein-based CSPs have had functional chromatographic lifetimes of only a few months. In addition, column costs have been substantial considering their short periods of usability. However, recent modifications in protein and chromatographic technologies coupled with a better understanding of the protein stationary phase have provided some new protein stationary phases with better separating capabilities and longer lifetimes.

In this study, several CSPs were investigated for their ability to separate the enantiomers of promethazine, ethopropazine, trimeprazine and trimipramine. The CSPs investigated were (*R*)-α-naphthylethylurea, (*S*)-leucine-(*R*)-α-naphthylethylamine and dinitrobenzoylphenylglycine (Pirkle-type columns); β-CD, β-acetylated CD, γ-CD, Chiralcel-OD, Chiralcel-OJ (inclusional); and Ovomucoid (affinity). Detection of the enantiomers was performed by fluorimetric analysis using excitation at 254 nm and a 280 nm emission filter.

2. Experimental

2.1. Apparatus

The HPLC system consisted of a pump (Model 2510; Varian, Walnut Creek, CA, USA), equipped with a 100-μl injector loop (Model 7125; Rheodyne, Cotati, CA, USA), a fluorescence detector set at excitation 254 nm with a 280 nm emission filter (Model Spectroflow 980; Kratos, Ramsey, NJ, USA), and an integrator (Model SP 4290; Spectra-Physics, San Jose, CA, USA).

2.2. Solvents and chemicals

Hexane, absolute methanol, methylene chloride, chloroform, acetonitrile, isopropanol (HPLC grade), phosphoric acid (concentrated), ethylene dichloride F.C.C. and monobasic potassium phosphate (reagent grade) were all obtained from J.T. Baker (Phillipsburg, NJ, USA). Ethanol 95% was purchased from University of Georgia Central Research Stores. Ethanol 200 proof was purchased from Midwest Grain Products (Weston, MO, USA). Triethylamine HPLC grade was purchased from Fisher (Fairlawn, NJ, USA). Trimipramine, trimeprazine, promethazine and trifluoroacetic acid (TFA) were purchased from Sigma (St. Louis, MO, USA). Ethopropazine was purchased from Aldrich (Milwaukee, WI, USA). (*R*)- and (*S*)-promethazine enantiomers used in this study were synthesized through crystallization using D- and L-dibenzoylated tartaric acid [1] and analyzed to be >99% (w/w) pure by HPLC and polarimetric analyses.

2.3. Columns

The columns used in this study were: KK-CARNU, (α-*R*-naphthyl)ethylurea (YMC, Wilmington, NC, USA), 10 cm × 4.6 mm, 5 μm; Sumichiral OA-4700, (*S*)-*tert.*-leucine-(*R*)-1-(α-naphthyl)ethylamine (YMC), 25 cm × 4.6 mm,5 μm; KK-CAPG, dinitrobenzoylphenylglycine (YMC) 10 cm × 4.6 mm, 5 μm; β,β-acetylated and γ-CDs (Astec, Whippany, NJ, USA), all 25 cm × 4.6 mm, 5 μm; Chiralcel-OD and -OJ (J.T. Baker), 25 cm × 4.6 mm, 5 μm and ES-OVM Ovomucoid (Mac-Mod Analytical, Chadds Ford, PA, USA), 15 cm × 4.6 mm, 5 μm. Equilibration of the columns was performed prior to each experiment using a minimum of 90 column volumes of mobile phase and test injections to determine chromatographic reproducibility.

2.4. Chromatographic conditions

All chromatography was performed at ambient temperature (23 ± 1°C) and at 1 ml/min unless otherwise stated. The reversed-phase mobile phases used were various mixtures of 0.01 *M* monobasic potassium phosphate buffers, 0.1% triethylamine, absolute methanol, isopropanol, acetonitrile and 95% ethanol with the pH adjusted to 4.0 with concentrated phosphoric acid unless otherwise indicated. All normal-phase mobile phases were various mixtures of hexane, methylene chloride, 1,2-dichloroethane, chloroform, absolute methanol, 95% ethanol, absolute ethanol, isopropanol, acetonitrile, TFA and glacial acetic acid (GAA). The mobile phases were all degassed by vacuum filtration prior to use. The concentration of solutions studied were 500 ng/ml of the racemic drug mixture dissolved in the appropriate mobile phase.

3. Results and discussion

The chemical structures of the analytes studied are shown in Fig. 1. The initial Pirkle CSPs studied were an *R*-α-naphthylethylurea (CARNU) with one chiral center and a (*S*)-*tert.*-leucine-(*R*)-1-(α-naphthyl) ethylamine (OA-4700) with two chiral centers. Baseline resolution of the enantiomers of all four compounds were achieved using these two columns. As shown in Table 1, promethazine enantiomers were successfully separated on the CARNU column with a resolution of at least 1.0 (baseline $R_s = 1.50$) using a wide variety of mobile phases. (This table reports only those mobile phases that provided a R_s of at least 1.0. Other mobile phases were used but were not listed for the sole purpose to save space.) The best resolution (2.25) was achieved using a mobile phase consisting of hexane–absolute ethanol–TFA (80:15:0.2, v/v). The strengths of the mobile phases that provided enantiomeric separations ranged from 0.45 to 1.55 based on the Snyder values of the solvent selectivity triangle. Obviously, there was no distinct mobile phase strength at which the promethazine enantiomers would separate. However, a majority of the mobile phases that provided resolutions of at least 1.0 were composed of approximately 60% hexane and 13–27% methylene chloride with the remainder being an alcohol and an acidic modi-

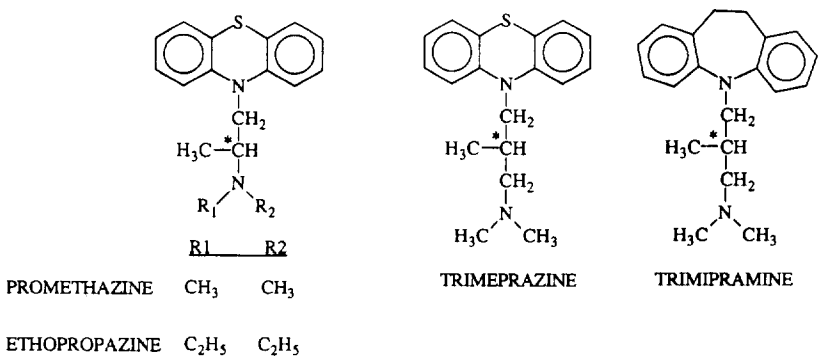

Fig. 1. Chemical structures of chiral compounds used in this study.

fier. In some cases, methylene chloride could be replaced by chloroform. This was important to enantiomeric separations on the CARNU column since methylene chloride is a dipole-interacting and chloroform a proton-donating solvent. However, chloroform was limited to no more than 25% of the total mobile phase composition. Nearly all of the mobile phases used either TFA or GAA as mobile phase modifiers to promote H bonding. Those mobile phases without either of the two acids had at least 15–20% alcohol to assist in H bonding.

Table 2 contains resolution and mobile phase data for other enantiomeric separations achieved on the CARNU column. Separation of the ethopropazine enantiomers was similar to that found for promethazine. However, ethopropazine is more lipophilic than promethazine, therefore, its enantiomers had different chromatographic interactions with the CARNU CSP

Table 1
Resolution of promethazine enantiomers on KK-CARNU stationary phase

Mobile phase composition (v/v)[a]									R_s	k'	
A	B	C	D	E	F	G	I	J		Peak 1	Peak 2
80					15		0.2		2.25	17.7	21.8
80		30			20		0.1		2.18	7.0	8.7
70	30			10			0.1		2.16	3.3	4.1
80	30				20		0.002		1.92	6.1	7.6
80		30			10		0.2		1.91	6.9	8.5
80			30		20		0.2		1.88	5.5	6.7
80	15	15			10		0.1		1.85	5.9	7.3
80	30				20		0.2		1.80	5.7	7.1
70	30	10					0.1		1.72	5.0	6.1
80	30				20				1.63	7.7	10.1
80			17	10			0.1		1.52	6.3	7.4
80		30			20			0.1	1.34	11.7	13.7
70	30					20			1.24	3.9	4.5
70	30			10		10			1.11	3.3	3.9
70	25					25			1.09	3.7	4.2
80	30				20			0.2	1.06	10.9	13.2
70	30					20	0.1		1.01	3.1	3.7

[a] A = Hexane; B = methylene chloride; C = chloroform; D = 1,2-dichloroethane; E = absolute methanol; F = absolute ethanol; G = 95% ethanol; I = trifluoracetic acid; J = glacial acetic acid.

Table 2
Resolution of ethopropazine and trimeprazine enantiomers on KK-CARNU stationary phase

Drug	Mobile phase composition (v/v)[a]									R_s	k'	
	A	B	C	D	E	F	G	I	J		Peak 1	Peak 2
Ethopropazine	80					15		0.2		1.89	20.1	23.5
	70	30			10			0.1		1.86	3.6	4.1
	80		30			20		0.1		1.69	8.2	9.5
	80			30		20		0.2		1.43	6.3	7.3
	80	30				20			1	1.36	15.7	18.0
	80	30				20		0.001		1.33	6.7	7.9
	80	30				20		0.2		1.31	6.4	7.4
	80			17	10			0.1		1.26	7.1	7.9
	80	30				20			0.2	1.23	10.6	12.2
	80	15	15			10		0.1		1.22	6.7	7.7
	70	30				10		0.1		1.20	5.4	6.2
	70	30					10	0.1		1.00	3.4	3.7
Trimeprazine	80					15		0.2		1.11	23.7	25.7

[a] Solvents as in Table 1.

and the mobile phases investigated. The result was a decrease in the total number of separations with resolutions of at least 1.0 for the ethopropazine enantiomers. Trimeprazine and trimipramine enantiomers were not baseline resolved on the CARNU column. Trimeprazine had only one enantiomeric separation with a resolution value greater than 1.0 and trimipramine enantiomers did not separate under any of the mobile phase conditions tested.

Substituting 1,2-dichloroethane for methylene chloride or chloroform in the mobile phase had little effect on enantiomeric resolution or peak shape, but 1,2-dichloroethane did change retention times on the CARNU column. This would be expected since methylene chloride and 1,2-dichloroethane are in the same Snyder solvent group, while chloroform is in a different group. Fig. 2 shows the separations of ethopropazine on the CARNU column using mobile phases containing methylene chloride and 1,2-dichloroethane. Other mobile phase changes included the substitution of absolute methanol, acetonitrile, isopropanol, 95% ethanol and absolute ethanol within a given mobile phase. Acetonitrile was not useful in providing an adequate fluorimetric environment for the detection of the

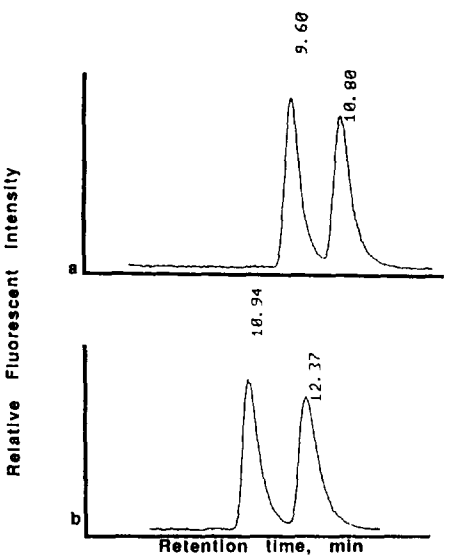

Fig. 2. (a) Typical HPLC chromatogram of the separation of ethopropazine enantiomers on CARNU column with hexane–methylene chloride–absolute ethanol–TFA (350:150:100:1, v/v) at 1 ml/min. (b) Typical HPLC chromatogram of the separation of ethopropazine enantiomers on CARNU column with hexane–1,2-dichloroethane–absolute ethanol–TFA (400:150:100:1, v/v) at 1 ml/min.

enantiomers of any of the four compounds. Isopropanol tended to decrease resolution as well as significantly reduce retention times when compared to all other solvents except methanol. For example, promethazine enantiomers were detected at 7.40 and 8.33 min ($R_s = 1.24$) using 95% ethanol on the CARNU column, but all of the resolution was lost with isopropanol and a single peak was obtained at a retention time of 5.56 min. Mobile phase additives such as TFA and GAA were tested. As a rule, TFA gave retention times approximately 50–60% that of GAA, while R_s with TFA was 1.1–1.7 times greater than GAA. The reader should take note that 0.1% of TFA has an *apparent* pH of 2.0 in a non-aqueous environment. However, the lifetimes of the Pirkle columns used in this study have been measured in years, therefore, it does not appear that the TFA does detrimental damage to the silica backbone of the stationary phase.

Results of enantiomeric separations on the OA-4700 column are shown in Table 3. Promethazine enantiomers were separated with a resolution of 1.70 using a mobile phase of hexane–methylene chloride–absolute ethanol–trifluroacetic acid (160:15:10:0.1, v/v) . Other separations of promethazine enantiomers with resolutions of at least 1.0 used mobile phases containing 1,2-dichloroethane rather than methylene chloride. Absolute methanol proved to be too polar for promethazine enantiomeric separations to occur; therefore, absolute ethanol was used. Small amounts of TFA were added to enhance H bonding. However, there were few separations with resolution values of at least 1.0 for promethazine enantiomers on the OA-4700 column. No separations with a resolution value of at least 1.0 occurred when absolute methanol, chloroform, isopropanol or GAA were components in the mobile phase. Using a wide variety of mobile phases on the OA-4700 column, resolution values of ethopropazine enantiomers were 0.70–0.99. Enantiomeric separations on the OA-4700 column for both trimeprazine and trimipramine yielded resolutions of at least 1.5

Table 3
Resolution of promethazine, trimeprazine and trimipramine enantiomers on OA-4700 stationary phase

Drug	Mobile phase composition (v/v)[a]					R_s	k'	
	A	B	D	F	I		Peak 1	Peak 2
Promethazine	160	15		10	0.1	1.70	3.4	4.2
	120		15	10	0.1	1.44	2.3	2.5
	160		15	10	0.1	1.25	3.6	3.9
	90		15	10	0.1	1.17	1.4	1.6
	80		15	10	0.1	1.11	1.2	1.4
Trimeprazine	160		15	10	0.1	2.62	3.9	4.6
	120		15	10	0.1	2.50	2.4	2.8
	90		15	10	0.1	1.71	1.5	1.8
	160	15		10	0.1	1.70	3.3	3.9
	80		15	10	0.1	1.51	1.3	1.5
	80	15		10		1.25	0.8	1.0
	60		15	10	0.1	1.10	0.8	0.9
	50		15	10	0.1	1.00	0.2	0.7
Trimipramine	160	15		10	0.1	2.00	3.5	3.9
	120		15	10	0.1	1.76	2.2	2.6
	90		15	10	0.1	1.26	1.5	1.6
	160		15	10	0.1	1.25	3.6	4.0

[a] Solvents as in Table 1.

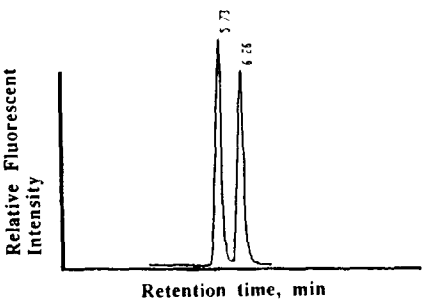

Fig. 3. Typical HPLC chromatogram of the separation of trimeprazine enantiomers on Sumichiral OA-4700 column with hexane–1,2-dichloroethane–absolute ethanol–TFA (800:150:100:1, v/v) at 1 ml/min.

with a variety of mobile phases. Figs. 3 and 4 show typical chromatograms for trimeprazine and trimipramine enantiomers on the OA-4700 column. Later calculations determined that the solvent strength of the mobile phase should be ≤ 0.90 in order for the trimeprazine and trimipramine enantiomers to separate on the OA-4700 column. In general, mobile phases with a solvent strength greater than 0.744 did not provide enantiomeric separations of promethazine, trimeprazine and trimipramine.

It was of interest that the enantiomers of promethazine and ethopropazine could easily be baseline resolved on the CARNU column with one chiral center, while trimeprazine and trimipramine enantiomers required the OA-4700 column with two chiral centers to achieve baseline separation. Both promethazine and ethopropazine have H-bonding sites α to the chiral centers; whereas trimeprazine and trimipramine

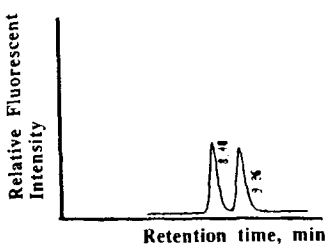

Fig. 4. Typical HPLC chromatogram of the separation of trimipramine enantiomers on Sumichiral OA-4700 column with hexane–1,2-dichloroethane–absolute ethanol–TFA (1200:150:100:1, v/v) at 1 ml/min.

have the H-bonding sites β to the chiral centers. The CARNU and OA-4700 stationary phases differ by the addition of a leucine amino acid moiety in the OA-4700 column backbone. It appears that the amino acid provides a specific spatial arrangement conducive to the separation of the enantiomers with their H-bonding centers β to the chiral center. Promethazine and ethopropazine do not require spatial rearrangement of the stationary phase's H-bonding center, therefore their enantiomers separated better on the CARNU column than on the OA-4700 column.

The energies associated with the various diastereomeric complexes for promethazine were low enough to allow the complexes to form, but the difference in diastereomeric complex energies was great enough for the enantiomers to separate. The ethopropazine enantiomers were unable to be separated on the OA-4700 column. Until modeling studies currently in progress in these laboratories are complete, this phenomenon is attributed to low complex energy differences due to increased lipophilicities associated with the N,N-diethyl groups of ethopropazine as opposed to the N,N-dimethyl groups on promethazine. Trimipramine has an ethylene bridge that replaces the sulfur bridge of a phenothiazine. The ethylene group causes the two phenyl rings of trimipramine to be almost perpendicular to each other, therefore the aromatic region of trimipramine is twisted. Trimipramine complexed with the stationary phase tended to have high Gibbs free energies with only minute differences between each one of the diastereomeric complex energies on the CARNU column. The high energies associated with the $\pi-\pi$ interacting region were overcome on the OA-4700 column and enantiomeric resolutions occurred.

The next column studied was the KK-CAPG column operated in the reversed-phase mode. Even though this CSP is not usually run in the reversed-phase mode, there was some evidence in the literature which showed that a dinitrobenzoylphenylglycine column was used in the reversed-phase mode with positive enantioselectivity [10,11]. However, the results from this work were not adequate. Peak symmetry was poor ($a/b \geq 2.0$) with no signs of enantiomeric

separations for any of the four compounds. To convert the CSP back to the normal-phase mode, considerable time and consideration was used for the proper selection of mobile phases in order to best readjust the stationary phase. Tests were run on the column using previously reported separations to determine if the CSP was adequately restored for normal-phase use. With the column operated in the normal-phase mode, separations were achieved for the phenothiazines, but the enantiomer peaks were only separated by 0.20 min, thereby giving an $R_s \leq 0.40$. No enantiomeric separations were obtained for trimipramine using this stationary phase. This is surprising since the stationary phase is strongly π-acidic and the compounds are π-basic in nature. The CSP must not be able to hold the $\pi-\pi$ interaction long enough for a difference in diastereomeric complex energies to occur.

Attempts to separate the enantiomers of promethazine, ethopropazine, trimeprazine and trimipramine using the three CD bonded phases were unsuccessful. Typical resolution values for the β and β-acetylated columns with a cavity opening of 7.8 Å across the wider opening were less than 0.45 and the peaks tended to be highly asymmetric ($a/b \geq 1.9$). However, the γ-CD column with a cavity opening of 9.5 Å across the wider opening did improve enantiomeric separations, but the resolution was ≤ 0.80. The γ-CD column was the most versatile and consistent CD column for the compounds studied since many of the mobile phases gave enantiomeric resolutions near 0.80.

According to Wainer and Drayer [8], there are a few basic structural rules that a compound should meet in order for the enantiomers to be separated on cyclodextrin stationary phases. These are: (1) the compound should not be so large that it cannot at least partially enter the CD cavity; (2) the chiral center should be near the mouth of the CD cavity; (3) the compound should have an aromatic system α or β to the chiral center, but occasionally the ring can be γ to the chiral center; and finally (4) there should be the potential for H bonding at or near the center for chirality. As seen in Fig. 1, the compounds promethazine, ethopropazine, trimeprazine and trimipramine all fit this description. However, the β,β-acetylated and γ-CDs did not allow the baseline separation of any of the four compounds. This was probably due to the size of the inclusion cavity. When modeling promethazine and trimipramine structures with the β- and γ-CD structures, our data showed that the basic three ring structures were almost entirely in high energy states. The γ-CD column, which gave better, but not baseline separations, has a cavity diameter and volume 1.22 and 1.67 times larger, respectively, than the β-CD cavity. This meant that the analytes were allowed to enter the γ cavity more freely than the β cavity. Yet, the enantiomeric distinction was not great enough in either case to allow baseline separations to occur for any of the compounds.

The cellulose-based stationary phases, Chiralcel-OJ [cellulose tris(4-methyl benzoate)] and Chiralcel-OD [cellulose tris(3,5-dinitrophenyl carbamate)] were investigated next. These columns were severely limited in the kinds of solvents that could be used in the mobile phase. Therefore, the mobile phases were either hexane–absolute ethanol or hexane–isopropanol mixtures. No enantiomeric baseline resolutions greater than 1.12 were detected for any of the racemic pairs using isopropanol on either cellulose column. While the Chiralcel-OD column did not provide enantiomeric separations with any of the mobile phases, the Chiralcel-OJ column provided separations ($R_s \geq 1.50$) for promethazine and near-baseline enantiomeric separations for ethopropazine and trimeprazine using hexane–absolute ethanol mobile phases as seen in Table 4. Fig. 5 shows a typical chromatogram for promethazine enantiomers on the Chiralcel-OJ column. Flow-rate analysis on these columns was performed at 0.5 and 0.8 ml/min, since the stationary phase is coated and not covalently bound. As expected, 0.8 ml/min gave better peak shapes, but there was no significant difference in resolutions for the same mobile phase at the two different flow-rates.

The enantioselective separations of the phenothiazines on the polymeric cellulose Chiralcel-OJ column is interesting in that it too consists of α-D-glucopyranose moieties, but not in a cyclic

Table 4
Resolution of promethazine, ethopropazine and trimeprazine enantiomers on Chiralcel-OJ stationary phase

Drug	Mobile phase composition (v/v)[a]			Flow-rate (ml/min)	R_s	k'	
	A	F	H			Peak 1	Peak 2
Promethazine	70	30		0.5	1.94	4.8	6.0
	90	10		0.5	1.88	6.9	8.8
	80	20		0.5	1.78	5.5	6.8
	80	20		0.8	1.70	3.0	3.9
	95	5		0.5	1.37	10.6	13.3
	95	5		0.8	1.21	6.1	7.8
	95		5	0.8	1.07	9.6	13.1
	90		10	0.5	1.01	9.9	12.6
Ethopropazine	80		20	0.5	1.04	2.9	4.5
Trimeprazine	90		10	0.5	1.12	7.4	9.0
	60	40		0.8	1.06	2.2	2.6
	50	50		0.5	1.06	4.0	4.6
	70	30		0.8	1.04	2.4	2.7

[a]A = Hexane; F = absolute ethanol; H = Isopropanol.

form. Detectable enantiomeric separations were obtained for promethazine ($R_s \leq 1.94$), trimeprazine ($R_s \leq 1.12$) and ethopropazine ($R_s \leq 1.04$) on the OJ column. This may be due to the planar characteristic of the phenothiazine tricyclic region versus the distorted and twisted tricyclic region of the dibenzazepine trimipramine. It is possible that the stereospecific tubes formed by the cellulose polymers are sterically limited; thus, making it difficult for the trimipramine enantiomers to enter the enantiodiscriminating cavity regions [13,14].

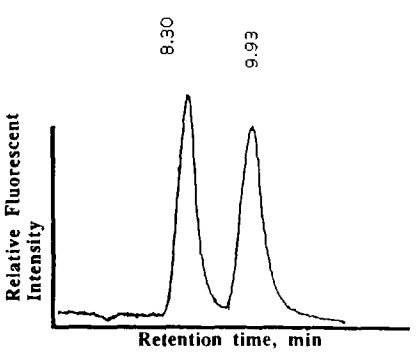

Fig. 5. Typical HPLC chromatogram of the separation of promethazine enantiomers on Chiralcel-OJ column with hexane–absolute ethanol (50:50) at 0.8 ml/min.

The last column investigated was the ovomucoid column from the affinity category. The poor enantioselective results of the four compounds on the ovomucoid column were possibly a result of the high affinity of these compounds for serum proteins. Promethazine enantiomers were separated with a resolution of 0.50, but none of the other compounds showed resolution. These results were interesting since all four compounds are highly protein bound in vivo (90–95%) [15]. It is possible that the proteinaceous stationary phase made little or no distinction between the enantiomers for these compounds; therefore, enantiomeric separations for these compounds require a different chiral environment than is provided by this stationary phase.

4. Conclusions

The CARNU and OA-4700 Pirkle-type CSP tended to provide more separations for large

aromatic compounds than the CAPG CSP. Typically, the CARNU and OA-4700 CSPs gave best results with approximately 60% hexane and 5–15% of an alcohol. A strong modifier such as TFA for basic compounds will sharpen peaks and tend to improve resolution. This allowed an investigator ample opportunity to select the remaining components and component ratios of the mobile phases in order to determine if chiral separations were possible for compounds of interest on that stationary phase. The Chiralcel-OJ CSP was able to separate the three phenothiazine compounds but did not show any tendency for an enantiomeric resolution for trimipramine. This indicated the channels composing the chiral separation centers in the cellulose CSP were not as strictly size limited as the CDs; rather, they were limited in the shape of the compound presented to the stationary phase. This Chiralcel-OD cellulose stationary phase would probably work best with analytes smaller than the ones studied here. Since there was little distinction between the bioactivity of each enantiomer of the compounds used in this study, it was possible that the highly protein bound nature of these compounds in vivo meant that there would be no distinction by protein CSP. Unless one enantiomer has a known bioactivity or therapeutic index different from the other enantiomer, a protein-based CSP would probably not be the starting point in trying to determine the proper stationary phase for a chiral resolution.

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