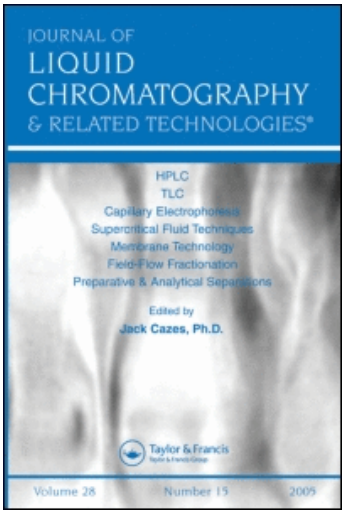


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# Pirkle-Concept Chiral Stationary Phases for the HPLC Separation of Pharmaceutical Racemates

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# PIRKLE-CONCEPT CHIRAL STATIONARY PHASES FOR THE HPLC SEPARATION OF PHARMACEUTICAL RACEMATES

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## ABSTRACT

The enantioselectivity of various Pirkle-concept chiral stationary phases (CSPs) for the direct HPLC separation of chiral drugs is explored and discussed. Seven different CSPs are surveyed for their utility to separate enantiomeric pairs belonging to the following major pharmaceutical classes: cardiovascular medicines (antihypertensives, antiarrhythmics, antianginals, diuretics), adrenergic drugs (vasopressors), antiinflammatory and analgesic compounds, topical anesthetics, antihistaminics, and antimalarial therapeutics.

## INTRODUCTION

The clinical, research and regulatory significance of chiral drugs continues to spur new developments into stereoselective methods of analysis. The majority of therapeutics administered today are both synthetic and racemic, despite well-documented differences in the pharmacodynamics and pharmacokinetics of the individual isomers.

One isomer can sometimes be many times more physiologically active and/or toxic than the other (1-3). Although the trend is to develop effective new therapeutic agents in optically pure form in order to fully exploit nature's "handedness", undoubtedly many drugs will continue to be developed as racemates for economic, technical or other reasons. Nevertheless, new regulatory guidelines (4) will make it necessary for the drug industry to evaluate the enantiomeric purity and toxicity/activity of any new drug introduction, including the development of "racemic switches" (5). Such driving forces necessitate that fast, sensitive and reproducible chromatographic methods be developed for the direct separation and quantitation of optical antipodes.



Enantiomeric separation by high performance liquid chromatography (HPLC) has taken various approaches over the last 25 years, with the most recent progress focused on the design and manufacture of totally synthetic chiral stationary phases, or CSPs (6-14). Such a wide variety of HPLC CSPs led Wainer to propose a classification scheme for choosing the most appropriate phase based on the structure of the solutes and the type of CSP, i.e., based on the chiral recognition mechanisms involved (15). The "three-point interactive rule" of chiral recognition first proposed by Dagliesh for paper chromatographic separations of amino acids (16) was later extended to HPLC and verified by Baczuk et al (17). Pirkle and his coworkers, however, began the first rational approach to the design of CSPs for HPLC using various optically active $\pi$-acids and $\pi$-bases and proved their wide utility (18-22). Resolution is achieved through a variety of diastereomeric interactions, including hydrogen bonding, dipole stacking, charge transfer, steric hinderance, etc . The enantiomer which forms the most energetically stable complex with the CSP is retained on the column longest. The "reciprocality

concept" put forth by Pirkle (20,22) allowed several generations of CSPs to evolve by analogy, including the many phases grafted by Oi and his group (23-32).

In this paper the utility of various CSPs of the type developed by Oi and coworkers are evaluated for their utility in resolving enantiomers of some important pharmaceutical compounds. Representative compounds of various drug classes, widely different in chemical structure, are surveyed and column enantioselectivity determined. General aspects of method development are also discussed.



## EXPERIMENTAL

### Chemicals

Analytical grade standards of all pharmaceuticals surveyed were obtained from Sigma (St. Louis, MO), Aldrich (Milwaukee, WI), or Fluka (Ronkonkoma, NY) chemical companies, depending on availability. Solutions for HPLC analysis were prepared in the range of 1 to 4 mg/ml by dissolving in hexane/ethanol (in various proportions, with ca 0.1% trifluoroacetic acid added) and sonicated as necessary. 99%+ reagent grade ammonium acetate and spectrophotometric grade trifluoroacetic acid were purchased from Aldrich. HPLC-grade solvents were obtained from either Fisher Scientific or Aldrich. All chemicals were used without further purification or filtration.

### Instrumentation

The Hewlett Packard 1050 HPLC system (Palo Alto, CA) consisted of a dual piston pump and solenoid valve proportioning system, multiple wavelength diode array-based detector with 8 $\mu$l flow cell, and a Rheodyne 7125 injector (Cotati, CA) equipped with a 20$\mu$l loop for full or partial injections from 2 to 20 $\mu$l. The optimal

detection wavelength for each compound was first determined by scanning the absorption spectrum from 200 to 400 nm using a Hewlett Packard 8452A diode array spectrophotometer. Data acquisition, storage and analysis were performed by the Hewlett Packard 1050 Chemstation software.

Chromatography

Table 1 lists the seven "brush" or Pirkle-concept CSPs used in the survey. All are commercially available as Chirex brand from Phenomenex, Inc. (Torrance, CA). Each CSP consists of an optically pure amino acid or carboxylic acid covalently bound to $\gamma$-aminopropyl silinized silica gel (5 $\mu$m particle size) and derivatized via an amide or urea linkage with a $\pi$-electron group. All columns were slurry-packed using a conventional technique into steel columns of standard 250 x 4.6 mm i.d. analytical dimensions and used without guard columns.

A variety of isocratic mobile phase systems were explored for their ability to enhance enantioseparation and control retention time. Two basic eluent compositions, a reversed phase system consisting of



TABLE 1

Chiral Stationary Phases

| Amino Acid | $\pi$-Electron Group | Linkage | Designation |
|---|---|---|---|
| (R)-phenylglycine | N-3,5-dinitrobenzoic acid | amide | 3001 |
| (R)-naphthylglycine | N-3,5-dinitrobenzoic acid | amide | 3005 |
| (S)-valine | (R)-1-($\alpha$-naphthyl)ethylamine | urea | 3014 |
| (S)-proline | (S)-1-($\alpha$-naphthyl)ethylamine | urea | 3017 |
| (S)-proline | (R)-1-($\alpha$-naphthyl)ethylamine | urea | 3018 |
| (S)-tert-leucine | (R)-1-($\alpha$-naphthyl)ethylamine | urea | 3020 |
| (S)-indoline-2-carboxylic acid | (R)-1-($\alpha$-naphthyl)ethylamine | urea | 3022 |

ammonium acetate in methanol or methanol/water, and a normal phase system containing hexane/ethanol/trifluoroacetic acid (with or without 1,2-dichloroethane), were used throughout. In all cases, retention times were primarily controlled by varying the concentration of alcohol. Similar eluents have been used with related Pirkle phases with good results (28,33), and these provided guidelines for the current study. Trifluoroacetic acid (TFA) was added to normal phase systems to improve peak shape. Premixed at a ratio of 1 part TFA to 20 parts ethanol, the final concentration of TFA in the mobile phase varied from 0.1-1.5%, and depended on the concentration of ethanol necessary to eluent all components while still preserving enantioseparation. Flow rates varied from 0.7 to 1.0 ml/min. Observed column pressures ranged from 600 to 1000 psi. All analytical runs were performed at ambient temperature.



## RESULTS AND DISCUSSION

### Optimizing the Separation

Ethanol concentration appeared to play a larger role than column selectivity in controlling retention time. The greatest determinant of enantioselectivity, on the other hand, was the CSP itself, rather than any manipulation of the mobile phase composition. Stereoselectivity of the CSP for the chiral solutes could quickly be evaluated by testing any given sample under conditions which eluted the peak or peaks within the first 5 to 15 minutes. If the required stereoselectivity, i.e., spatial complimentarity and stereospecific interaction, was present, separation would be observed within this time frame. Further optimization of the separation would then be pursued. Additional changes in mobile phase composition (within the parameters chosen) did little to bring about separation if selectivity was not already apparent.

## TABLE 2
## *Separation Factors for Racemic Drugs*

| Compound Name | Clinical or Therapeutic Use | UV abs Max (nm) | Column Enantioselectivity Separation Factor Alpha* Chiral Stationary Phase | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 3001 | 3005 | 3014 | 3017 | 3018 | 3020 | 3022 |
| Acebutolol | Antihypertensive | 244 | 1 | 1 | 1 | P D | 1 | 1 | 1.09 D |
| Atropine | Anticolinergic | 258 | | | 1 | | | 1 | 1 |
| Bendroflumethiazide | Diuretic | 272 | 1.11 D | 1.07 F | 1.14 F | P B | 1.09 D | 1.17 F | P F |
| Bepridil | Antianginal | 250 | 1 | | 1.22 R | 1 | 1.16 D | 1.09 L | P J |
| Brompheniramine | Antihistaminic | 264 | | | 1.30 B | | | 1.15 G | P D |
| Captopril | Antihypertensive | 242 | 1 | 1 | | | | | |
| Carbinoxamine | Antihistaminic | 262 | | | 1.15 C | | | 1.10 G | P D |
| Chloramphenicol | Antibacterial | 272 | | | 1 | | | 1 | 1 |
| Chloroquine | Antimalarial | 346 | 1 | 1 | 1.22 B | P A | 1.20 A | 1.21 B | 1.13 A |
| Chlorthalidone | Diuretic | 240 | | | P J | | | 1.08 K | 1 |
| Clemastine | Antihistaminic | 236 | 1 | 1 | 2.86 D | 1 | 1.26 B | 3.04 D | 1.35 F |
| Clenbuterol | Bronchodilator | 248 | 1 | 1 | 1 | 1 | 1.15 B | P L | 1.27 B |
| Colchicine | Gout suppressant | 352 | | | 1 | | | 1 | 1 |
| Cyclopentolate | Mydriatic | 260 | | | 1.13 J | | | 1.10 G | 1.07 D |
| Dichloroisoproterenol | Bronchodilator | 272 | | | 1.06 L | | | 1 | 1.13 J |
| Diltiazem | Antianginal | 248 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Diperodon | Anesthetic, local | 242 | | | 1.20 G | | | 1.09 L | P G |
| Disopyramide | Antiarrhythmic | 262 | 1 | 1 | 1 | 1 | P G | 1 | 1 |
| Doxylamine | Bronchodilator | 262 | | | P D | | | 1.07 G | |
| Ephedrine | Bronchodilator | 258 | | | 1 | | | 1 | P D |
| Epinephrine | Bronchodilator | 282 | | | 1 | | | 1.07 D | 1.10 D |
| Glafenine | Analgesic | 346 | 1 | 1 | P D | 1 | 1 | P D G | 1 |
| Hydroxy-phenyl-5-phenyl-hydantoin | Anticonvulsant | 238 | | | 1 | | | 1.11 | 1 |
| Hydroxyphenylethylamine | Vasopressor | 258 | | | 1 | | | 1 | |
| Hydroxyzine | Antihistaminic | 238 | 1 | | 1 | 1 | P F | 1 | 1 |
| Indapamide | Antihypertensive | 248 | 1 | 1 | 1 | 1 | P F | 1 | 1.08 F |
| Indoprofen | Analgesic, NSAID | 284 | 1 | 1.08 U | | | | | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Isoetharine | Bronchodilator | 282 | | | | | P | F | | | | 1.21 | D | 1.08 D |
| Isoproterenol | Bronchodilator | 282 | | | | | 1.08 | E | | | | 1.21 | D | 1.18 D |
| Isoxsuprine | Vasodilator | 276 | | | | | 1.40 | D | | | | 1.37 | D | P G |
| Labetolol 1 | Antihypertensive | 308 | | | | | P | D | | | | 1.08 | G | 1.08 D |
| Labetolol 2 | Antihypertensive | 308 | | | | | 1 | | | | | 1.22 | G | 1.11 D |
| Mephenesin | Skeletal muscle relaxant | 272 | 1 | | 1 | | 1 | | 1 | | 1 | | 1 | |
| Metanephrine | Vasopressor | 280 | | | | | 1 | | | | 1 | | 1.10 | D |
| Metaproterenol | Bronchodilator | 278 | | | | | 1.10 | E | | | | 1.24 | D | 1.13 D |
| Methocarbamol | Skeletal muscle relaxant | 274 | | | | | 1.09 | L | | | | 1.09 | G | 1 |
| Methotrimeprazine | Analgesic | 254 | 1 | | | | 1 | | 1 | | 1 | | 1 | |
| Methoxamine | Vasopressor | 290 | | | | | 1.10 | K | | | | 1.11 | G | 1.11 D |
| Methoxyverapamil | Antiarrhythmic | 278 | | | | | P | L | | | | | | 1.15 D |
| Metoprolol | Antihypertensive | 276 | | | | | P | M | | | | 1 | | 1.08 G |
| Midodrine | Adrenergic stimulant | 292 | 1.09 | D | 1 | | 1.09 | F | 1 | | 1 | | 1.10 | F 1 |
| Nadolol | Antihypertensive | 270 | 1 | | 1 | | 1 | | 1 | | P | F | 1 | P F |
| Nefopam | Analgesic | 268 | 1 | | 1 | | P | R | 1 | | 1.14 | B | P | G 1.10 F |
| Nicotine | Antismoking therapeutic | 260 | | | | | 1.10 | B | | | | | P | G P D |
| Normetanephrine | Vasopressor | 280 | | | | | | | | | | | 1 | P D |
| Octopamine | Vasopressor | 276 | | | | | 1 | | | | | | 1 | P D |
| Oxybutynin Chloride | Anticolinergic | 258 | | | | | 1 | | | | | | 1 | 1 |
| Oxyphencyclimine | Anticolinergic | 244 | 1 | | 1 | | 1 | | 1.50 | A | 1 | | 1 | 1 |
| Phenylbutazone | Antiinflammatory | 246 | 1 | | 1 | | 1 | | 1 | | 1 | | 1 | 1 |
| Phenylethanolamine | Vasoconstrictor, topical | 224 | | | | | 1 | | | | | | | 1 |
| Prilocaine | Anesthetic, local | 242 | | | | | 1.13 | J | | | | | 1.17 | G 1.14 G |
| Primaquine | Antimalarial | 268 | 1 | | | | 1.18 | D | 1 | | 1 | | 1.16 | F 1 |
| Primidone | Anticonvulsant | 240 | 1 | | 1 | | 1 | | 1 | | 1 | | 1 | 1 |
| Proglumide | Anticolinergic | 242 | 1.27 | D | 1.45 | U | 1.17 | R | | | 1 | | 1 | 1 |
| Promethazine | Antihistaminic | 254 | | | | | 1.12 | J | | | | | 1.12 | G 1.08 K |
| Propranolol | Antiarrhythmic | 292 | | | | | 1.11 | H | | | | | 1.08 | K 1.10 K |
| Quinacrine | Antimalarial | 282 | 1 | | 1 | | P | D | 1.38 | A | P | A | 1.10 | D 1.33 A |
| Sulpiride | Antidepressant | 242 | | | | | 1 | | | | | | 1 | 1 |
| Suprofen | Analgesic, NSAID | 288 | 1 | | P | U | | | | | | | | |
| Suxibuzone | Antiinflammatory | 246 | 1 | | 1 | | 1 | | 1 | | 1 | | 1 | 1 |
| Synephrine | Vasopressor | 276 | | | | | 1 | | | | | | 1 | 1.12 D |
| Turbutaline | Bronchodilator | 278 | | | | | 1.11 | J | | | | | 1.28 | D 1.24 D |
| Terfenadine (Seldane) | Antihistaminic | 260 | 1 | | | | 1 | | 1 | | 1 | | P | L P F |
| Tetrahydrozoline | Adrenergic stimulant | 242 | 1 | | | | 1.11 | D | 1.28 | B | 1.07 | B | 1.07 | D 1.12 B |
| Thioridazine | Antipsychotic | 264 | 1 | | | | P | R | | | 1 | | P | L 1.10 F |
| Timolol | Antihypertensive | 298 | 1 | | 1 | | 1 | | 1 | | 1 | | 1 | 1 |

(continued)

## TABLE 2 (continued)
## Separation Factors for Racemic Drugs

| Compound Name | Clinical or Therapeutic Use | UV abs Max (nm) | Column Enantioselectivity Separation Factor Alpha* Chiral Stationary Phase | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 3001 | 3005 | 3014 | 3017 | 3018 | 3020 | 3022 |
| Tropicamide | Anticolinergic | 254 | 1 | 1 | P G | 1 | 1 | 1 | 1.10 D |
| Verapamil | Antiarrhythmic | 280 | | | 1.10 N | | | 1.05 L | 1.11 G |
| Warfarin | Anticoagulant | 282 | | | 1 | | | 1 | 1.03 P |

* Alpha (α) = K2/K1; P = Partially separated; Blank denotes no separation attempted. Mobile phase conditions are indicated by letter codes following alpha values for all complete or partial separations and are explained at the bottom of the table.

Normal Phase Solvent Systems:

| | Hexane (%) | 1,2-Dichloro-ethane (%) | Ethanol-TFA† (%) |
|---|---|---|---|
| A | 40 | 35 | 25 |
| B | 50 | 35 | 15 |
| C | 40 | 50 | 10 |
| D | 55 | 35 | 10 |
| E | 43 | 50 | 7 |
| F | 58 | 35 | 7 |
| G | 60 | 35 | 5 |
| H | 70 | 25 | 5 |
| I | 73 | 20 | 7 |
| J | 75 | 20 | 5 |
| K | 76 | 20 | 4 |
| L | 77 | 20 | 3 |
| M | 82 | 15 | 3 |
| N | 87 | 10 | 3 |
| O | 92 | 5 | 3 |
| P | 88 | 10 | 2 |
| Q | 78 | 20 | 2 |
| R | 62 | 35 | 3 |

Reversed Phase Solvent Systems:

| | Ammonium Acetate (M) | Methanol (%) | Water (%) |
|---|---|---|---|
| S | 0.005 | 100 | 0 |
| T | 0.01 | 100 | 0 |
| U | 0.025 | 100 | 0 |
| V | 0.03 | 100 | 0 |
| W | 0.05 | 100 | 0 |

† Note: Ethanol and TFA (trifluoroacetic acid) were pre-mixed 20:1.

An initial survey of most compounds was performed at 25, 15, or 10% ethanol, and 35 or 20% 1,2-dichloroethane, with hexane making up the remainder. Some of the more polar test compounds could be sufficiently retained only by dropping the concentration of ethanol down to 1-3%. Phases used with this system were 3001, 3014, 3017, 3018, 3020 and 3022. Phase 3005 appeared well-suited to the analysis of carboxylic acids and amides and was evaluated under both normal and reversed phase conditions. In normal phase mode, various combinations of hexane and ethanol (premixed with TFA) were tried with some success. Reversed phase systems consisting of 25mM ammonium acetate in methanol/water mixtures also proved effective.



The capacity of these low-molecular weight CSPs for separating and purifying a wide variety of enantiomers has been amply demonstrated (23-32). The current survey significantly expands the known applications of these phases. The selection of compounds was designed to be representative of the diverse classes of pharmaceuticals which are either being developed enantiomerically pure, or converted in a "racemic switch", because of reduced side effects and/or enhanced potency.

Table 2 summarizes the efforts to separate and optimize the resolution of 70 racemic pharmaceuticals of wide structural variety using seven different CSPs. At least 24 clinical/therapeutic drug categories are represented. Not all compounds were tested on all CSPs. Percentages of successfully resolved enantiomers (Table 3) ranged from 10% to 45% per CSP. Enantiomer peaks were considered resolved if baseline or valley between the pair dropped to less than 25% of the height of the first peak. When partially and fully resolved enantiomers are combined together, the percentages ranged

from 10% to 65%. Successful resolution of all compounds on all columns was 60%, and the total of successful and partial separations achieved was 80%; only 20% of the selected compounds showed no stereospecific interaction with the tested CSPs.

Separations of Cardiovascular Drugs

The β-adrenergic blocking agents labetolol and nadolol both contain multiple stereogenic centers. Phase 3020 successfully resolved all four isomers of labetolol (Figure 1), a compound which has been administered for hypertension as both a racemate and partially purified, but was later shown to cause liver toxicity (5).



TABLE 3

Separation Utility of Seven Pirkle-Concept CSPs

| | 3001 | 3005 | 3014 | 3017 | 3018 | 3020 | 3022 | ALL |
|---|---|---|---|---|---|---|---|---|
| S | 3 | 4 | 24 | 3 | 7 | 29 | 29 | 46 |
| P | 0 | 1 | 11 | 3 | 5 | 6 | 13 | 10 |
| T | 31 | 24 | 66 | 26 | 28 | 64 | 65 | 70 |
| %S | 10 | 17 | 36 | 12 | 25 | 45 | 45 | 60 |
| %S+P | 10 | 21 | 53 | 23 | 43 | 55 | 65 | 80 |

S = Separated enantiomeric pair (baseline or valley height between the pair is less than 25% of the height of peak one).

P = Partial separation of the enantiomeric pair (valley height is greater than 25% of the height of peak one).

T = Total number of compounds tested.

Nadolol, containing three chiral centers, was partially resolved into three peaks on phases 3018 and 3022. The best result was obtained on the latter phase and is shown in Figure 2. Superior enantioresolution of methoxyverapamil, one compound in the widening class of calcium channel modulators, was achieved in under 7 minutes on phase 3022 (Figure 3). Six of the seven test columns showed at least partial stereoselectivity for the diuretic bendroflumethazide (Table 2). Peak resolution obtained on phase 3001 is shown in Figure 4.

Separations of Antiinflammatory and Analgesic Compounds

Initial chiral HPLC methods for the analysis of non-steroidal antiinflammatory drugs (NSAIDs) required prior derivatization. Derivatization with chiral reagents is prone to error (34) and achiral derivatization (35-36), while providing detection benefits, can complicate the chromatography. Unpublished data from Oi and coworkers, and, more recently, unpublished data from Jamali's group, have shown the wide utility of phase 3005 for the direct resolution of NSAID enantiomers. In Figure 5 the separation of indoprofen enantiomers using this phase is presented. Figure 6 shows the enantioseparation of nefopam, another useful analgesic drug, on phase 3018.

Separations of Adrenergic Compounds

The separations of two adrenergic-stimulating amino alcohols, synephrine and tetrahydrozoline, are shown in Figures 7 and 8, respectively. Relatively few chiral chromatography systems have shown the capacity for direct enantioresolution of these compounds (37) Because their pharmacological effects reside primarily in the (-)-

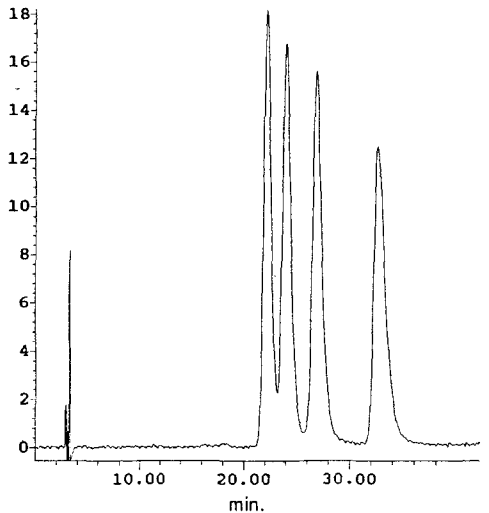


FIGURE 1. Chromatographic separation of racemic Labetolol (β-adrenergic receptor blocker) on Chirex phase 3020. The mobile phase was hexane/1,2 dichloro-ethane/ethanol-trifluoroacetic acid (60:35:5, with ethanol-TFA premixed 20:1) at a flow rate of 1.0 ml/min. UV detection was used at 308 nm. Assignments of the four isomers were not known.

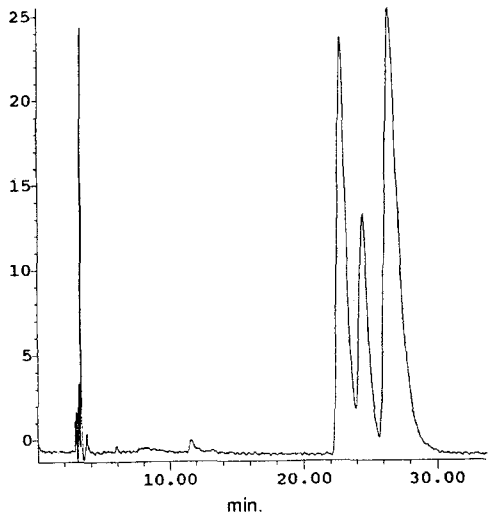


FIGURE 2. Chromatographic separation (partial) of racemic Nadolol (β-adrenergic receptor blocker) on Chirex phase 3022. The mobile phase was hexane/1,2-dichloroethane/ethanol-trifluoroacetic acid (58:35:7, with ethanol-TFA premixed 20:1) at a flow rate of 1.0 ml/min. UV detection was used at 270 nm.

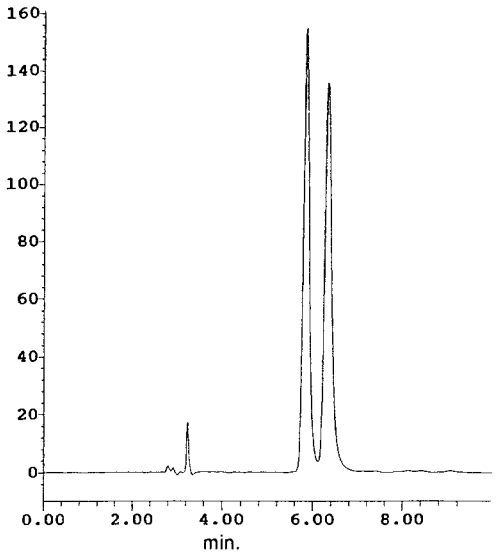


FIGURE 3. Chromatographic separation of racemic Methoxyverapamil (calcium channel modulator) on Chirex phase 3022. The mobile phase was hexane /1,2-dichloroethane/ethanol-trifluoroacetic acid (55:35:10, with ethanol-TFA premixed 20:1) at a flow rate of 1.0 ml/min. UV detection was used at 278 nm. $\alpha = 1.15$

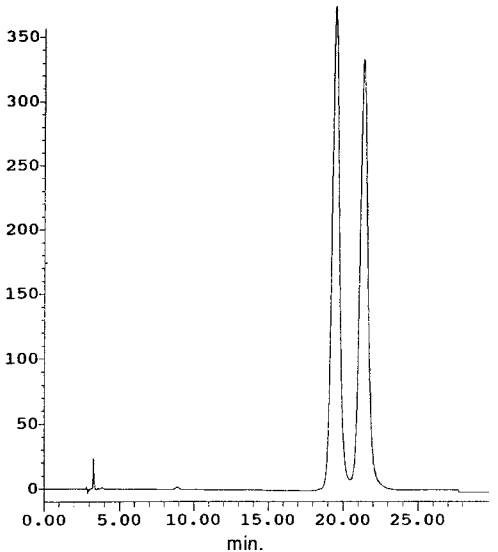


FIGURE 4. Chromatographic separation of racemic Bendroflumethazide (diuretic) on Chirex phase 3001. The mobile phase was hexane/1,2-dichloroethane/ ethanol-trifluoroacetic acid (55:35:10, with ethanol-TFA premixed 20:1) at a flow rate of 1.0 ml/min. UV detection was used at 272 nm. $\alpha = 1.11$

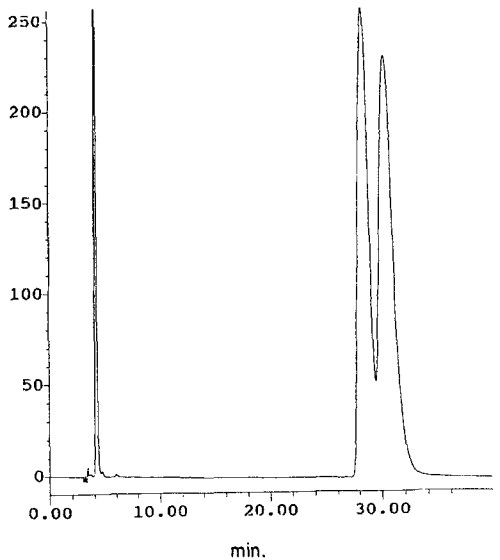


FIGURE 5. Chromatographic separation of racemic Indoprofen (NSAID) on Chirex phase 3005. The mobile phase was 0.025 M ammonium acetate in 100% methanol at a flow rate of 1.0 ml/min. UV detection was used at 284 nm. $\alpha = 1.08$

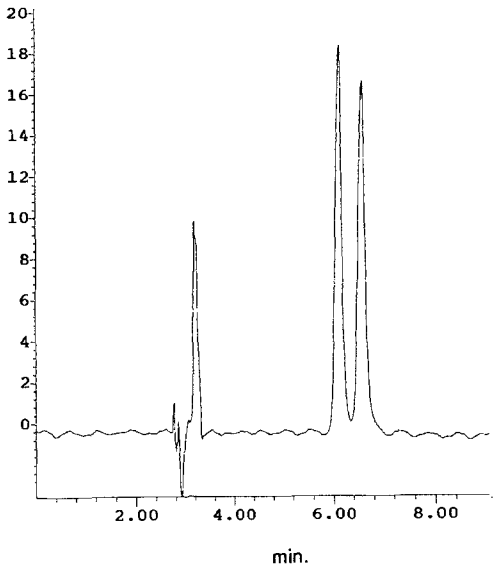


FIGURE 6. Chromatographic separation of racemic Nefopam (analgesic) on Chirex phase 3018. The mobile phase was hexane/ 1,2-dichloroethane/ethanol-trifluoroacetic acid (50:35:15, with ethanol-TFA premixed 20:1) at a flow rate of 1.0 ml/min. UV detection was used at 268 nm. $\alpha = 1.14$

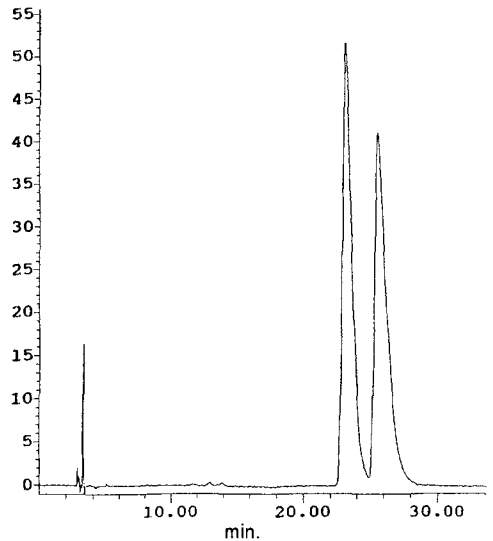


FIGURE 7. Chromatographic separation of racemic Synephrine (vasopressor) on Chirex phase 3022. The mobile phase was hexane/1,2-dichloroethane/ethanol-trifluoroacetic acid (55:35:10, with ethanol-TFA premixed 20:1) at a flow rate of 1.0 ml/min. UV detection was used at 276 nm. $\alpha = 1.12$

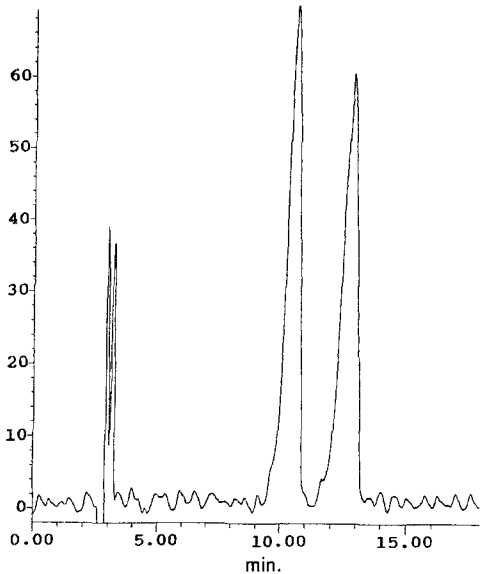


FIGURE 8. Chromatographic separation of racemic Tetrahydrozoline (β-adrenergic stimulant) on Chirex phase 3017. The mobile phase was hexane/1,2dichloro-ethane/ethanol-trifluoroacetic acid (50:35:15, with ethanol-TFA premixed 20:1) at a flow rate of 1.0 ml/min. UV detection was used at 242 nm. $\alpha = 1.28$

(R)-enantiomers, the unique stereoselectivity of these columns will be of benefit. Note the successful resolution of tetrahydrozoline on several of the test CSPs (Table 2).

Separations of Antihistaminic Drugs

Of the six antihistaminic drugs tested in this survey, four were well-resolved on several columns and two were partially resolved (Table 2). Separations of two compounds are shown in Figures 9 and 10. The differences in pharmacological activity and disposition of the pheniramine derivatives, chlor- and brompheniramine, are well known (38), but attempts to resolve and study the enantiomers by chiral chromatography have met with limited success (39). These CSPs now extend the current analytical capabilities.

Separations of Antimalarial Therapeutics

The enantiospecific differences between antipodes of the antimalarial drugs is another area of active investigation (40). Three compounds were chosen for testing in the current study: chloroquine, primaquine and quinacrine. The excellent separations of all three compounds obtained on several CSPs should prove to be valuable analytical tools for investigating their different pharmacodynamic and pharmacokinetics properties.

Separations of Topical Anesthetics

Two topical anesthetics were selected for the current survey. Both appeared to have similar stereoselectivity on three of the columns, but no resolution on the other four. Optimum results for the chiral separations of diperodon and prilocaine are shown in Figures 13 and 14.

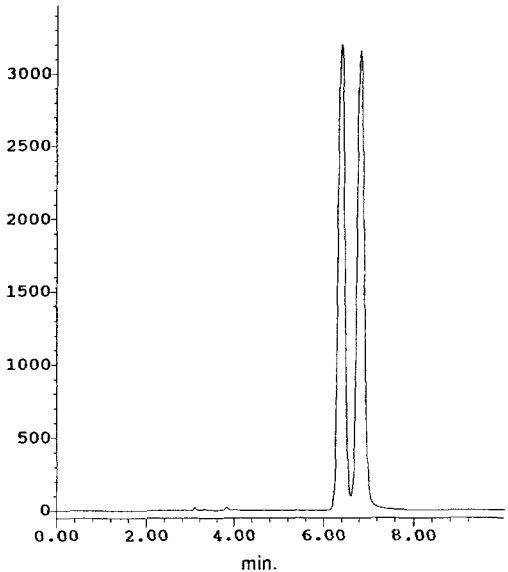


FIGURE 9. Chromatographic separation of racemic Promethazine (antihistaminic) on Chirex phase 3020. The mobile phase was hexane/1,2-dichloroethane/ ethanol-trifluoroacetic acid (60:35:5, with ethanol-TFA premixed 20:1) at a flow rate of 1.0 ml/min. UV detection was used at 254 nm. $\alpha = 1.12$

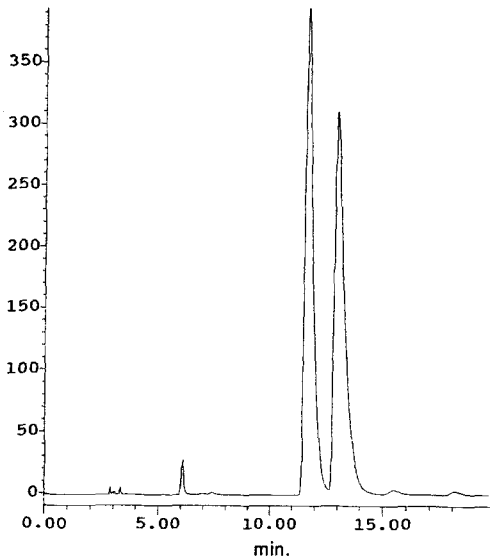


FIGURE 10. Chromatographic separation of racemic Brompheniramine (antihistaminic) on Chirex phase 3020. The mobile phase was hexane/1,2-dichloroethane/ethanol-trifluoroacetic acid (60:35:5, with ethanol-TFA premixed 20:1) at a flow rate of 1.0 ml/min. UV detection was used at 264 nm. $\alpha = 1.15$

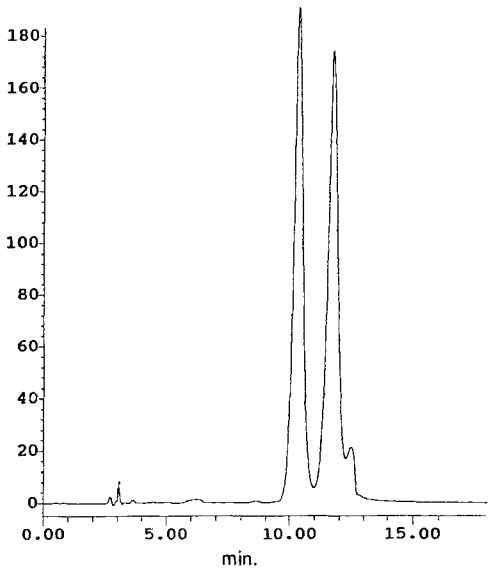


FIGURE 11. Chromatographic separation of racemic Primaquine (antimalarial) on Chirex phase 3014. The mobile phase was hexane/1,2-dichloroethane/ethanol-trifluoroacetic acid (55:35:10, with ethanol-TFA premixed 20:1) at a flow rate of 1.0 ml/min. UV detection was used at 268 nm. $\alpha = 1.18$

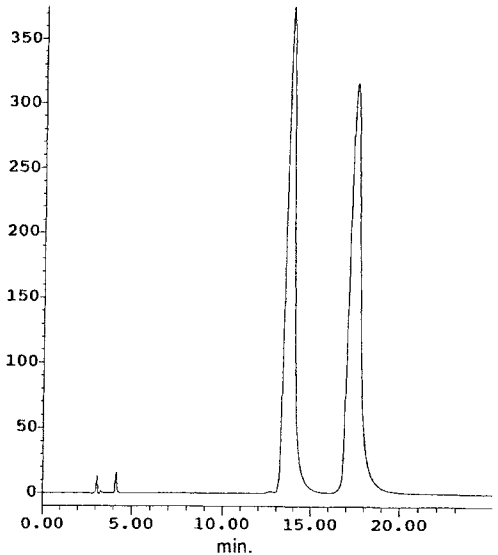


FIGURE 12. Chromatographic separation of racemic Quinacrine (antimalarial) on Chirex phase 3022. The mobile phase was hexane/1,2-dichloroethane/ethanol-trifluoroacetic acid (40:35:25, with ethanol-TFA premixed 20:1) at a flow rate of 1.0 ml/min. UV detection was used at 282 nm. $\alpha = 1.33$

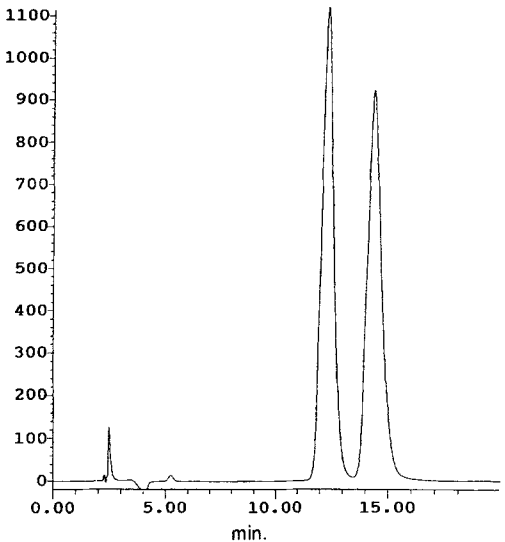


FIGURE 13. Chromatographic separation of racemic Diperodon (local anesthetic) on Chirex phase 3014. The mobile phase was hexane/1,2-dichloroethane/ethanol-trifluoroacetic acid (60:35:5, with ethanol-TFA premixed 20:1) at a flow rate of 0.7 ml/min. UV detection was used at 242 nm. $\alpha = 1.20$

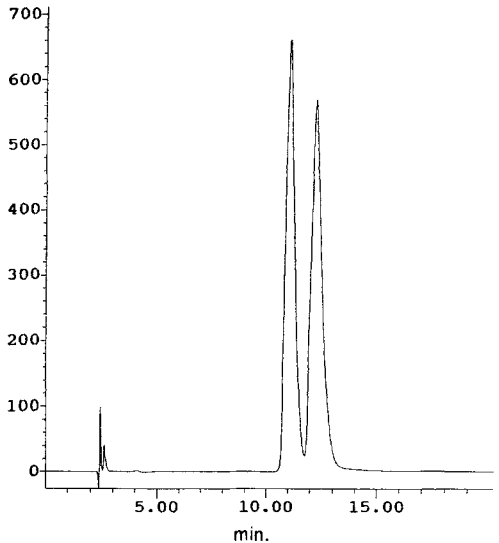


FIGURE 14. Chromatographic separation of racemic Prilocaine (local anesthetic) on Chirex phase 3014. The mobile phase was hexane/1,2-dichloroethane/ethanol-trifluoroacetic acid (75:20:5, with ethanol-TFA premixed 20:1) at a flow rate of 0.7 ml/min. UV detection was used at 242 nm. $\alpha = 1.13$

## CONCLUSION

The large number of possible interactions (41-42) provided by the multiple chemical functionalities contained in each CSP is what gives these types of columns their broad applicability. Compared with other, naturally-derived CSPs, these low-molecular weight phases demonstrate considerably higher efficiencies, are robust and long-lasting, and more suitable for quantitative work (7,9). Some columns in the current study have been actively used for more than one year without any perceptible degradation in performance (unpublished data). Reversed phase separations are possible using several phases in this class, but separations performed under normal phase conditions often afford greater enantioselectivity (14,43 ).



Although chiral separations are known to be highly compound-specific with respect to any given CSP (6-7,9,13,42,44), the resolution of a wide variety of racemic pharmaceuticals was clearly demonstrated in the current study using a select number of Pirkle-concept phases. An easy approach to rapid method development was presented. High-efficiency packings and exceptional CSP selectivities combined to produce baseline separations for the majority of test compounds. The application of these phases to the areas of chiral drug synthesis, analysis in biological fluids and enantiomeric purity determinations will make these CSPs an excellent choice for chiral separations and aid in the development of superior therapeutics.

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