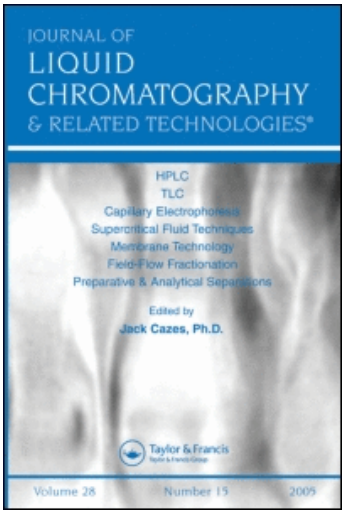


**Journal of Liquid Chromatography & Related Technologies**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# Selection of the Mobile Phase for Enantiomeric Resolution via Chiral Stationary Phase Columns

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**To cite this Article** Zief, M. , Crane, L. J. and Horvath, J.(1984) 'Selection of the Mobile Phase for Enantiomeric Resolution via Chiral Stationary Phase Columns', Journal of Liquid Chromatography & Related Technologies, 7: 4, 709 — 730
**To link to this Article: DOI:** 10.1080/01483918408073997
**URL:** http://dx.doi.org/10.1080/01483918408073997

JOURNAL OF LIQUID CHROMATOGRAPHY, 7(4), 709-730 (1984)

# SELECTION OF THE MOBILE PHASE FOR ENANTIOMERIC RESOLUTION VIA CHIRAL STATIONARY PHASE COLUMNS

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## ABSTRACT

The optimization of enantiomeric resolution by mobile phase variation was studied with the chiral stationary phase derived from R-N-(3,5-dinitrobenzoyl)phenylglycine covalently coupled to 5 μm spherical 3-aminopropyl silica. Chromatography was routinely performed with mobile phase compositions having polarities as high as 2.5 without column deterioration. The relative strength of a solvent as a hydrogen acceptor was found to be an important basis for selection of the polar component in a binary mobile phase. The substitution of tert-butanol for 2-propanol or ethanol in an alcohol/hexane mixture, for example, afforded improved separation factors with several enantiomers. In addition, the need for a polar mobile phase such as 50/50 methylene chloride/hexane to minimize non-specific polar absorption of enantiomers has been demonstrated. Enhancement of specific chiral interactions and suppression of interfering reactions have been obtained with a number of clinically relevant derivatives as model compounds.

## INTRODUCTION

The design of chiral stationary phases for the chromatographic separation of enantiomers has been a



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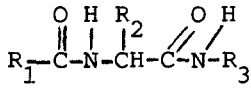
major focus of interest in a number of laboratories over the past several years. Originally the chiral stationary phases were developed for gas chromatographic separations. More recently, specific optically active phases have been used in the HPLC mode as well.

Although a variety of approaches has been used, one very important class of optically active phases in both the HPLC and GC mode has been derived from a chiral diamide functional group (I). This diamide group contains multiple hydrogen bond donor and acceptor groups[1]. In addition, restricted rotation around the amide bond affords a preferred face for interaction with one of a pair of enantiomeric solutes.



(I)

Some of the most effective optically active phases in GC studies are based upon naturally occurring amino acids such as S-valine, in which $R_2$ = $-CH(CH_3)_2$. Attachment to the solid support occurs either through the amine $R_3$ or the carboxyl $R_1$. The same approach is being used with considerable success in HPLC. In this case either $R_1$ or $R_3$ represents an attachment to silica via a bridging group; most commonly $R_3$ represents n-propyl silica. This structure is most often synthesized by treating the carboxyl group of the chiral amino acids with 3-aminopropyl silanized silica.

Perhaps the most extensive investigation of enantiomeric separations on HPLC chiral stationary phases of

this type has been conducted by Pirkle and his colleagues, who have developed several variations of (I) in which $R_1$ is 3,5-dinitrophenyl, $R_2$ is phenyl or isobutyl and $R_3$ is propyl-silanized silica[2]. These phases have thus been derived from α-R-phenylglycine or S-leucine, by bonding N-3,5-dinitrobenzoyl derivatives of these chiral amino acids to 3-aminopropyl silanized silica either covalently via an amide linkage or ionically via acid-base interactions. Both of these types of chiral stationary phases are now commercially available.



Although investigators differ somewhat in the details of interpreting bonding forces required for chiral resolution (recognition), it is generally agreed that differential binding of enantiomers results from differences in the summation of binding energies of hydrogen bond acceptor and donor groups, π bonding and steric interactions. Hydrogen bonding has long been recognized as a contributing interaction; Pirkle has more recently proposed, with his stationary phase, that dipole stacking plays an important role[3]

Whatever the specific types of interaction involved between the enantiomers and the chiral stationary phase, it is clear that any competing interaction may alter enantiomeric resolution. In GC, where the mobile phase is an inert carrier gas, the possibility of polar interaction with chiral and non-chiral groups is minimal. In HPLC, however, the mobile phase is a dynamic part of the system and must be recognized as a potential source of

polar functional groups capable of interacting with both the chiral stationary phase and with the enantiomeric solute. The approach of most investigators has been to mimic GC conditions by using a mobile phase that is as "inert" as possible (i.e., non-polar) while possessing sufficient solubilizing ability to move the enantiomer through the HPLC column. Most commonly, hexane with small amounts of isopropanol (3-10%) has been the preferred mobile phase. We have taken a somewhat different approach, basing our studies upon the proposition that the mobile phase for chiral chromatography can be utilized, as in other modes of HPLC, to optimize both enantiomeric resolution and overall chromatographic quality of any chiral separation. The results that we have obtained with a number of clinically relevant derivatives as model compounds are presented in this paper.



## MATERIALS AND METHODS

### Apparatus

The chromatography was performed with an Altex 110 high-pressure pump, a Gilson Model 111 UV-detector set at 254 nm and a Hewlett-Packard strip-chart recorder.

The HPLC column used was a BAKERBOND Chiral Phase™ DNBPG (covalent), from J. T. Baker Chemical Company, a standard 25 cm x 4.6 mm I.D. stainless steel HPLC column packed with a bonded phase of R-N-3,5-(dinitrobenzoyl)-phenylglycine covalently coupled to 5 µm spherical 3-aminopropyl silica.

## Materials

The β-naphthamide of amphetamine and the 3,4-dimethyl-2(-2-naphthyl)-5-phenyloxazolidine were obtained from Dr. E. Wainer, Food and Drug Administration, Washington, DC. Dr. W. H. Pirkle, University of Illinois, supplied the 7-chloro-1,3-dihydro-3-benzyl-5-phenyl-1,4-benzodiazepin-2-one. The 1-methoxy-3(1[-2-nitroimidazole])-propanol-2 was furnished by Dr. J. L. Day, Florida A&M University. All the solvents were J. T. Baker HPLC grade.



## Derivatives

Preparation of N, and N,O-α-naphthoyl derivatives was carried out by a modification of the procedure of Pirkle and Welch[3].

## Propranolol Derivatives

Propranolol hydrochloride (0.1 g) and α-naphthoyl chloride (0.1 g) were added to 5 mL of methylene chloride in a 20-mL vial. To this mixture was added 3 mL of 5% aqueous sodium hydroxide solution. The vial was capped and shake vigorously for one minute. The aqueous layer was removed with a Pasteur pipet. The lower organic layer was washed once with 3% hydrochloric acid, then twice with distilled water, and was finally dried over anhydrous sodium sulfate. The filtered organic layer was directly injected onto the HPLC column.

## DISCUSSION

Mobile phases for chiral columns are usually binary mixtures of solvents. For such mixtures the polarity

($P^1$) is readily obtained from the following equation:

$$P^1 = \phi_A P_A + \phi_B P_B$$

Where $O_A$ and $O_B$ are the volume fractions of solvents A and B and $P_A$ and $P_b$ are the $P^1$ values of the pure solvents[4].

As can be seen in Table 1, the (0-10%) IPA-hexane mobile phases used most frequently in published studies of the R-N-3,5-dinitrobenzoylphenylglycine column range in polarity from 0.2-0.48. This low polarity enhances weak chiral interactions, as emphasized previously, but also has been considered necessary to prevent leaching of the ionically bound chiral phase from the column (leaching reported at a polarity of 0.805[5]). More recently, columns have become available with the chiral amino acid covalently bound to the silica support via an amide linkage. Chromatography with the covalent columns has been routinely performed with methylene chloride/ hexane mixtures at polarities as high as 2.5 with no column deterioration. Additionally, the covalent columns have been extensively washed with water ($P^1$ = 10.2) with no deterioration. Thus far, no combination of solutes and solvents routinely used in normal phase chromatography has altered the structure of the covalent stationary phase. Furthermore, strongly retained adsorbates can be removed from the column rapidly with methanol.

The increased stability of the covalent stationary phase has made it possible to investigate mobile phase

TABLE I

Polarity ($P^1$) of Binary Mobile Phases

| Mobile Phase | Solvent A 2-Propanol, % | Solvent B Hexane, % | $P^1$ |
|---|---|---|---|
| | | 100 | 0.1 |
| 1 | 5 | 95 | 0.29 |
| 2 | 10 | 90 | 0.48 |
| 3 | 20 | 80 | 0.85 |
| 4 | 30 | 70 | 1.24 |
| 5 | 50 | 50 | 2.00 |
| 6 | 100 | | 3.9 |
| | Methylene Chloride, % | Hexane, % | |
| 7 | 50 | 50 | 1.60 |
| 8 | 70 | 30 | 2.20 |
| 9 | 80 | 20 | 2.50 |
| 10 | 100 | | 3.1 |

composition as a variable in optimizing chiral chromatography. The need to explore mobile phase selectivity became apparent when simple variation of 2-propanol/hexane binary mixtures failed to resolve the N,O-α-naphthoyl derivatives of propranolol, even though resolution would be predicted based upon the present hypotheses for selective interaction.

Snyder has proposed that solute retention in liquid-solid chromatography on polar adsorbents can be explained by a comprehensive model based on solvent adsorption onto and subsequent displacement from sites on the adsorbent by molecules of solute[6]. According to this hypothesis, the relative ease of displacement of solvent on the adsorbent by solute will largely determine the retention time of the solute. If there are specific localized sites on the adsorbent, these will be most important in solvent adsorption for small increments of interactive solvents. Therefore in our system one should be able to compare solvent adsorption for interactive solvents in binary mixtures by measuring relative elution times for specific optical isomers at the same solvent polarity (same solvent strength). A decrease in elution time would signify that solvent molecules adsorbed onto active sites of the chiral phase are displaced with greater difficulty by solute molecules.



Snyder's hypothesis is general for polar adsorbents, including silica, alumina, and bonded phase sorbents such as amino alkyl, and includes less site specific solvent-adsorbent interactions as well. For chiral phase I where the π-acidic 3,5-dinitrophenyl group is $R_1$, the acidic amide hydrogen offers the possibility for specific interaction for basic solvents, i.e., solvents having hydrogen acceptor capabilities.

To see whether an interaction of this nature is important, we referred to the solvent selectivity triangle of Snyder[7] and selected for replacement of

2-propanol (a hydrogen donor-acceptor) in the 2-propanol/hexane mixture, ethyl ether (a hydrogen acceptor), chloroform (a pure hydrogen donor) and methylene chloride (a poor hydrogen donor or acceptor, but possessing a large dipole moment). In addition, tetrahydrofuran and ethyl acetate, intermediate between ethyl ether and methylene chloride were investigated.

Two compounds were chosen as model solutes for most of our studies; 2,2,2,-trifluoro-1-(-9-anthryl)ethanol, weakly retained by the column but well-resolved because of its clearly defined sites for specific interaction, including a strongly interactive alcohol moiety; and the α-naphthamide of 1-(α-naphthyl)ethylamine, quite strongly retained by the stationary phase by dipole-dipole interaction and π-π bonding.

The data in Table II showing relative elution times support the concept that the relative strength of the solvent as a hydrogen acceptor is an important basis for solvent adsorption. With solute A and a mobile phase polarity of 0.48, relative elution times place solvent adsorption onto the chiral phase in the following order: 2-propanol > tetrahydrofuran > ethyl acetate > ethyl ether > methylene chloride > chloroform. These data agree with the hydrogen accepting ability reported by Taft, et al, who listed hydrogen-acceptor constants ($pK_{HB}$) of 1.26 for tetrahydrofuran, 1.08 for ethyl acetate, and 0.98 for ethyl ether[8]. N-Butylamine ($pK_{HB}$ = 2.11) is so strong an acceptor that it may form a tertiary complex with the solute on the stationary phase and therefore retard elution.

TABLE II

Evaluation of Mobile Phases for Enantiomeric Resolution

Solute A: 2,2,2,-trifluoro-1(-9-anthryl)ethanol

| Mobile Phase | $P^1$ | $t_1$ | $t_2$ | $\alpha$ | $k_1^1$ |
|---|---|---|---|---|---|
| 5/95 n-butylamine/hexane | 0.30 | solute retained beyond 20 minutes | | | |
| 10/90 +-butanol/hexane | 0.50 | 3.8 | 5.1 | 1.62 | 1.23 |
| 10/90 2-propanol/hexane | 0.48 | 3.6 | 4.6 | 1.56 | 1.0 |
| 9/91 ethanol/hexane | 0.48 | 3.8 | 4.5 | 1.33 | 1.23 |
| 10/90 tetrahydrofuran/hexane | 0.49 | 6.1 | 8.1 | 1.47 | 2.38 |
| 9/91 ethyl acetate/hexane | 0.49 | 8.5 | 11.9 | 1.50 | 3.72 |
| 14/86 ethyl ether/hexane | 0.48 | 12.0 | 17.0 | 1.49 | 6.06 |
| 40/60 methylene chloride/hexane | 1.30 | 15.6 | 21.7 | 1.44 | 7.66 |
| 50/50 methylene chloride/hexane | 1.60 | 6.0 | 8.0 | 1.48 | 2.33 |

| | | | | | |
|---|---|---|---|---|---|
| 70/30 methylene chloride/hexane | 2.20 | 3.5 | 4.1 | 1.42 | 0.94 |
| 60/40 chloroform/hexane | 2.50 | 5.0 | 6.1 | 1.33 | 1.94 |

Solute B: α-naphthamide of 1-(α-naphthyl)ethylamine

| | | | | | |
|---|---|---|---|---|---|
| 30/70 2-propanol/hexane | 1.24 | 8.7 | 13.8 | 1.73 | 4.12 |
| 40/60 methylene chloride/hexane | 1.30 | 16.8 | 23.7 | 1.46 | 8.88 |
| 50/50 methylene chloride/hexane | 1.60 | 10.0 | 13.9 | 1.48 | 4.88 |
| 70/30 methylene chloride/hexane | 2.20 | 5.1 | 6.6 | 1.44 | 2.0 |

Conditions: 25 cm x 4.6 mm covalent chiral column.

Flow Rate: 2 mL/min.

2-Propanol and other alcohols are uniquely effective solvents as mobile phase additives with respect to solute A, which is itself an alcohol. As the 2-propanol content in the mobile phase increases, displacement of the solvent by solute A becomes more and more difficult. It appears that the hydroxyl group of solute A competes directly with the hydroxyl group of 2-propanol for specific sites on the stationary phase. The first isomer of solute A elutes at 3.6 minutes with 10/90 2-propanol/hexane, at 2.6 minutes with a 20/80 ratio and 2.3 minutes with a 30/70 ratio, the effect diminishing as adsorption sites are saturated.



When methylene chloride is substituted for 2-propanol in the mobile phase, the solute can then displace the adsorbed solvent readily. Removal of the solute from the chiral stationary phase then requires a polarity of 2.2 in order to attain the $k_1^1$ value (approximately 1.0) observed with 10/90 2-propanol/hexane ($P^1 = 0.48$). When chloroform replaces methylene chloride in the mobile phase, removal of the solute demands a mobile phase of still higher polarity. These data support the hypothesis that a hydrogen donor solvent is most easily displaced from the chiral stationary phase.

The structure of solute B would predict adsorptive interactions of a far less site specific, geometrically constrained nature, and indeed, though 2-propanol is still a more effective solvent than methylene chloride, its unique effectiveness in minimizing retention times is

muted. Whereas the elution times $t_1$ for solute A differ by a factor of 6.8 for 30/70 2-propanol/hexane and 40/60 methylene chloride/hexane, they differ by only a factor of 1.9 for solute B. Here the high solvent strength of the methylene chloride binary mobile phase becomes an important factor.

## Variation of Enantiomeric Separation, α, with the Mobile Phase

Chromatographic separation of enantiomers on a chiral stationary phase depends solely upon the differential ability of the two optical isomers to form very specific, transient, geometrically constrained complexes with a small number of adjacent sites on the stationary phase. For this reason, although retention times change as the composition (and therefore polarity) of a particular binary mixture changes, the separation factor stays relatively constant (see Figure 1). Most of the "site specific" interactions previously discussed as retention time determinants affect both enantiomers relatively equally; i.e., they are not chiral in nature.

The separation factor does increase, however, as the tendency of the active solvent to interact specifically with the chiral moiety on the stationary phase increases. Thus as Table II shows, tertiary butanol and 2-propanol give higher α values for solute A than do the other solvents capable of acting as hydrogen acceptors, which as a group have higher α values than does the single hydrogen donor solvent chloroform. The same trend is indicated for solute B.

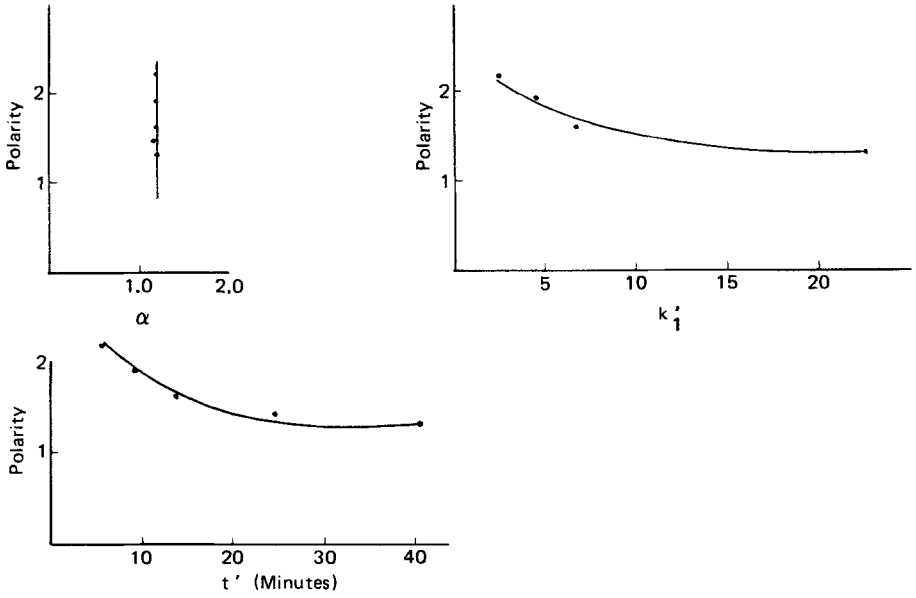



Figure 1

Behavior Of Propranolol ∝ - Napththamide In Methylene Chloride/Hexane Mobile Phases Flow Rate: 2 ml/min.

Of the three alcohols tested with solute A, t-butanol gives the highest α value. As the alcohol increases in bulk, both enantiomers are able to displace solvent molecules more readily. The more tightly bound isomer, the S-form, introduces more desolvation of the stationary phase than the R-form, resulting in an increase in α due primarily to an increase in the retention time of the more tightly bound enantiomer.

The bulkiness of the solvent molecule seems to be an important factor contributing to larger α values with ethers also. Of course, ethers are primarily hydrogen

acceptors whereas alcohols are able to donate hydrogen bonds as well. If bulkiness is important, one would predict that the combination of t-butyl methyl ether/-hexane as a mobile phase should not only have lower retention times because of its greater basicity than a corresponding ethyl ether/hexane mixture of equal polarity, but also have a higher α value. Indeed, as Table III shows, when solute A was chromatographed in a mobile phase of a 17/83 t-butyl methyl ether/hexane mixture (P = 0.49), the retention times were approximately half those for an equivalent ethyl ether/hexane mixture, and the α value approached the value obtained with 2-propanol as the interactive solvent. The highest α value (2.12) was obtained by accident, when solute A dissolved in 50/50 2-propanol/ hexane was chromatographed in 17/83 t-butyl methyl ether/hexane. The retention time of the first enantiomer was sharply reduced, reflecting the transient effect of the small amount of 2-propanol in the system, while the retention time of the second enantiomer was nearly the same as in the mobile phase alone.



## Application of Mobile Phase Selectivity to Enhance Enantiomeric Resolution

Our working hypotheses in solvent selection were tested with a variety of enantiomeric pairs in an attempt to optimize resolution. Table IV lists a number of such compounds. Compound C is a β-naphthyl oxazolidine derivative of ephedrine, originally suggested by Wainer (9) as a means to achieve chromatographic enantiomeric

TABLE III

Evaluation of t-Butyl Methyl Ether/Hexane Mobile Phase

Solute A: 2,2,2,-trifluoro-1-(9-anthryl)ethanol

| Mobile Phase | $P^1$ | $t_1$ | $t_2$ | $\alpha$ | $k_1^1$ |
|---|---|---|---|---|---|
| [a]17/83 t-butyl methyl ether/hexane | 0.49 | 6.3 | 8.8 | 1.54 | 2.7 |
| [b]17/83 t-butyl methyl ether/hexane | 0.49 | 4.8 | 8.3 | 2.12 | 1.82 |
| 14/86 ethyl ether/hexane | 0.48 | 12.0 | 17.0 | 1.49 | 6.06 |

(a) Injection of solute dissolved in the mobile phase.

(b) Injection of solute dissolved in 50/50 2-propanol/hexane.

resolution of this biologically important compound. This derivative is particularly interesting because of its low retention on the chiral phase chromatographed in the traditional 5/95 2-propanol/hexane mobile phase. When this solvent (P = 0.29) was used in our laboratory, the k' value was 0.73; α was 1.07. When the mobile phase was changed to 14/86 diethyl ether hexane (P = 0.48) retention was prolonged (k' = 2.2) and better resolution of the peaks was observed.

Propranolol is another hydroxy amine that can form several derivatives. Condensation with α-naphthoyl chloride gives the N and the N,O-substituted naphthamides (I) containing two and three naphthoyl residues, respectively. With 2-propanol/hexane mobile phases, the N-naphthamides are easily resolved. In this binary solvent mixture, however, the N,O-derivatives, are retained inordinately long at low polarity (P = 0.29) and elute together with no separation at high polarity (P = 1.24). When the polarity is reduced to 0.86, separation occurs, but retention times are still inconveniently long. At polarities sufficiently high to give convenient retention times, adsorption of 2-propanol on the chiral phase obviates resolution of the bulky N,O-derivatives. Simple replacement of 2-propanol with methylene chloride solves the problem. Table IV shows that at a polarity of 2.2 the separation is complete within 12 minutes.

Compound H is a good example of the need for a polar mobile phase selected to minimize non-specific polar adsorption of the enantiomers onto the stationary phase.

TABLE IV

Separation of Racemic Mixtures on a Covalent R-N-(3,5-Dinitrobenzoyl)phenyl Glycine Chiral Stationary Phase[1]

| Solute | Chemical Name | Mobile Phase[2] | $t_1$ | $t_2$ | $k'_1$ | $\alpha$ | $t_3$ | $t_4$ | $\alpha$[5] |
|---|---|---|---|---|---|---|---|---|---|
| C | 3,4-dimethyl-2(β-naphthyl)-5-phenyloxazolidine | 1 | 10.9 | 11.5 | 0.73 | 1.07 | | | |
| D | 7-chloro-1,3-dihydro-3-benzyl-5-phenyl-2H-1,4-benzodiazepin-2-one | 1 | 8.0 | 19.0 | 3.44 | 2.77 | | | |
| E | β-naphthamide of amphetamine | 1 | 17.8 | 19.2 | 9.47 | 1.09 | | | |
| F | α-naphthamide of 1(α-naphthyl)ethylamine | 4 | 8.7 | 13.8 | 4.1 | 1.73 | | | |
| G | 1-(α-naphthyl)ethylamine | 4 | 13.3 | 14.3 | 6.8 | 1.09 | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| H | 1-methoxy-3(2-nitro-imidazole)propanol-2-α-naphthoate | 8 | 6.9 | 8.5 | 3.06 | 1.31 | | | |
| I | N and N,O-α-naphthoyl derivatives of propranolol | 2 | >25 | | | | | | |
| | | 3 | 9.4 | 10.3 | 4.5 | 1.12 | 15 | 16 | 1.08 |
| | | 4 | 7.5 | 8.3 | 3.4 | 1.14 | 11 | 11 | 1.00 |
| | | 5 | 4.5 | 5.1 | 1.6 | 1.20 | 7.5 | 7.5 | 1.00 |
| | | 7 | 13.8 | 16.0 | 6.6 | 1.18 | 25.92 | 29.0 | 1.11 |
| | | 8 | 5.8 | 6.4 | 2.4 | 1.15 | 10.51 | 11.6 | 1.13 |
| | | 9(3) | 4.0 | 4.0 | | | | | |
| | | 10(4) | 1.7 | 1.7 | | | | | |

(1) Flow rate of mobile phase: 0.5 mL/min with compound C; 2 mL/min with other ocmpounds.
(2) See Table I.
(3) No resolution.
(4) Solute appears at solvent front.
(5) Values of $t_3$, $t_4$ and α for N,O-α-naphthoyl derivatives of propranolol.

The nitroimidazole group may bind to the stationary phase and prolong elution of the enantiomers. At a relatively high polarity (2.2), however, methylene chloride provides excellent resolution within 9 minutes. Resolution disappears if 2-propanol is a component of the mobile phase.

Compound G is particularly interesting because it contains an amine group as part of the chiral center. Traditionally underivatized amines have not been chromatographed on this type of chiral column because the relatively non-polar mobile phases used failed to elute such compounds from the column. To solve this problem the hydrogen donor properties of 2-propanol are necessary for complexing with the amine group and thus reducing the attachment of the free amine to the chiral phase. At a solvent ratio of 30/70 2-propanol/hexane, the enantiomers resolve with an $\alpha$ value of 1.09. In this case derivatization is desirable for increasing resolution as well as decreasing the relatively strong binding of the original $NH_2$ group. Compound F elutes in the same mobile phase with an $\alpha$ value of 1.73; k' decreases from 6.8 to 4.1. Derivatization is not always feasible if compounds must be recovered but it is frequently a simple way to dramatically enhance resolution.

Compound E (amphetamine) is closely related to F inasmuch as both compounds are naphthamides of 1-substituted ethyl amines. The 1-naphthyl substituent in G is replaced by the benzyl group in amphetamine. Since the $\pi$-basicity of the benzyl group is lower than that of naphthyl, weaker interaction of E with the 3,5-dinitro-

benzoyl fragment of the stationary phase is expected[3]. For optimum resolution, therefore, compound E should be exposed to a mobile phase of lower polarity than that (1.24) used with F. Even at the low polarity of 0.29, however, the separation factor for E was reduced to 1.09.

Finally, it is encouraging to include in this listing of challenging compounds a pharmaceutically interesting molecule (of the benzodiazepam family), compound D, that resolves in the classical mobile phase with an impressive $\alpha$ value (2.77) and reasonable elution times. This compound contains a substituted amide as part of a seven-membered ring system. The large $\alpha$ value can be attributed to the destabilization of the weaker diastereomeric chiral amino acid solute complex by the benzyl group.



## SUMMARY

An increasing number of studies are being published on enantiomeric separation via chiral stationary phases in LC. The two approaches previously reported to increase the applicability of this technique include derivatization of the enantiomers to enhance resolution or increase elutability, and alteration of the chiral moiety on the stationary phase. We have presented a third approach, manipulation of the mobile phase, on a DNBPG chiral column, to enhance specific chiral interactions while minimizing interfering or non-productive interactions. This approach should assist in optimizing chromatography for any enantiomeric pair intrinsically capable of resolution on a DNBPG chiral column.

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