It is also observed that the steady-state gain of the system is dependent on the size of the pulse strength. For linear operating conditions, the steady-state gain of the transfer function should remain independent of the input pulse size. Research into a normalized input pulse strength is currently under way.

Proposed uses for transfer functions include phenomenological modeling, process control, and FDA validation. To be useful with phenomenological modeling, non-linearities within the system must be linearized over known dependent variable ranges. It is our goal that phenomenological models for individual systems can be verified using empirical transfer functions. Breaking down a transfer function into classical chromatographic principles also holds potential for the further identification of chromatography. A validated phenomenological model could then be used as a tool for scale-up calculations. Frequency response results utilized for process control would be a direct application of transfer functions. Automatic-control system design for chromatography would benefit greatly from easily obtainable transfer functions to represent the operational systems.

If non-linearities persist, and specific contributions from the time domain cannot be related to transfer functions, pulse testing still offers a viable mechanism to uniquely "fingerprint" a chromatographic system. This "fingerprint" could be used to verify the proper operation of the system, much like statistical process control techniques used today. As an actual pulse test on a system is not time or material restrictive, pulse testing could be used to standardize chromatographic processes and thus be an ideal mechanism to enhance FDA validation procedures.

Acknowledgements

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Nomenclature

dB	decibel
G	transfer function
Im	imaginary part of transfer function
i	ith frequency
j	jth component of system order
j	imaginary number
K_p	steady-state gain
MR	magnitude ratio
n	system order
NFC	normalized frequency content
Re	real part of transfer function
s	Laplace variable
t	time (s)
T_x	input pulse time (s)
T_y	output pulse time (s)
x	input variable (mg/ml) in time domain
X	transformed input in frequency domain
y	output variable (mg/ml) in time domain
Y	transformed output in frequency domain
ξ	damping coefficient
θ_a	time delay analogue (s)
θ_d	time delay (s)
ϕ	phase angle (rad)
τ	break point (s/rad)
ω	frequency (rad/s)

References

[1] D.F. Bruley and J.W. Prados, AIChE J., 11 (September) (1964) 612.
[2] S.J Lees and J. Hougen, J. Ind. Eng. Chem., 48 (1956) 1064–1068.
[3] C.I.J. Lewis, D.F. Bruley and D.H. Hunt, Ind. Eng. Chem. Process Des. Dev., 6 (July) (1967) 281.
[4] W.L. Luyben, Process Control, McGraw-Hill, New York, 1991, pp. 502–534.
[5] W.C. Clements, Jr. and K.B. Schnelle, Jr., Ind. Eng. Chem. Process Des. Dev. 2, No. 2 (April) (1963) 94–102.
[6] F.H. Arnold, H.W. Blanch and C.R. Wilke, Chem. Eng. J., 30 (1985) B25–B36.
[7] R.D. Whitley, J.M. Brown, N.P. Karajgikar and N. Wang, J. Chromatogr., 483 (1989) 263–287.

[8] J.L. Wade, A.F. Bergold and P.W. Carr, Anal. Chem., 59 (1987) 1286–1295.
[9] M.Z. El Fallah, S. Golshan-Shirazi and G. Guiochon, J. Chromatogr., 511 (1990) 1.
[10] K.A. Kang, D.F. Bruley, J.M. Londono and B. Chance, Ann. Biomed. Eng., 22 (1994) 240–252.
[11] D.R. Coughanowr, Process Systems Analysis and Control, McGraw-Hill, New York.
[12] L.N.G. Filon, Proc. R. Soc. Edinburgh, 49 (1928) 38.
[13] D.F. Bruley, Filon's Method Data Reduction Code, DuPont, 1975, unpublished.

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Optimization of chiral selectivity on cellulose-based high-performance liquid chromatographic columns using aprotic mobile-phase modifiers☆

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Abstract

Tris-3,5-dimethyl phenyl carbamate-derivatized cellulose HPLC columns and other cellulose-based columns are popular for the direct separation of many racemic drugs and agrichemicals. These columns exhibit good efficiency and stability under well-controlled chromatographic conditions. Cellulose-based chiral columns also exhibit high sample-load capacity with normal-phase solvents, which is particularly advantageous for isolating purified solutes. Several investigators have reported that the stereoselectivity of these columns can be varied by changing the type and composition of alcohol modifiers in the mobile phase, and by altering temperature. We now find that separation selectivity and resolution can be significantly increased, and often optimized, by modifying the nonpolar mobile phase with certain aprotic solvents. The potential of this new separation dimension greatly increases the general applicability of cellulose-based columns for analytical and preparative separations of racemates. A simple, systematic scheme is proposed for optimizing the separation of enantiomeric drugs with the Chiralcel OD column.

1. Introduction

The HPLC separation of chiral compounds in pharmaceutical and agrichemical products is increasing in popularity because of regulatory and other concerns. Typically, useful HPLC separations of racemic mixtures are developed by scouting columns with different types of chirally-active stationary phases. However, such columns have proliferated to the point that it is difficult to chose a satisfactory mobile phase/column combination without considerable effort. Columns for analytical and preparative separations include the "Pirkle" type, ligand exchangers, inclusion-formation systems such as the cyclodextrins, various polysaccharide-based materials, and those based on various immobilized proteins. Systematic studies have defined the areas of best applications for these column types, and the experimental approaches for satisfactory results [1–5].

Previous studies in this laboratory have extensively utilized a silica-based column with an ovomucoid protein stationary phase (Ultron ES-OVM) for the analytical separation of a wide range of racemic drugs [6–8]. This column type often exhibits superior separations of racemic drugs with widely-differing characteristics [6]. Studies involving the effects of varying sepa-

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ration parameters with this column have led to development of simple and systematic schemes for optimizing the separation of enantiomeric agents [7,8]. Unfortunately, however, immobilized protein columns all suffer from an inherent disadvantage: sample loadings must be kept small or the column will overload, degrading separation performance. This characteristic of immobilized protein columns seriously limits this column type for separations where purified enantiomers must be isolated. Limitations in the protein-based columns directed us to study cellulose-based columns for achieving both analytical and preparative separations. Although preparative separations are not a subject of this report, methods for optimizing separations of racemic mixtures are analogous for both analytical and preparative applications.

Cellulose-based columns have several potential advantages over protein-based columns for separating racemic mixtures. First, cellulose-based columns have higher sample loading capacity. Second, the low-boiling organic solvents typically used with these columns facilitate the isolation of purified solutes. Totally organic solvent systems used with cellulose-based columns are readily removed from purified fractions, simplifying problems with removing the buffered aqueous mobile phases used with protein-based columns. Third, the cellulose-based columns are broadly applicable to many compound types [4]. Fourth, commercially-available short columns of these materials rapidly equilibrate and allow faster separations for decreased method development time. Lastly, short cellulose columns are less expensive, permitting the practical survey of a range of column types at reasonable cost.

However, practical disadvantages of the cellulose-based columns had to be overcome in this study. There are many different types of cellulose-based chiral columns [9]. Which type might be the most promising so that the testing of all might not be required? Also, the chromatographic performance of cellulose-based columns is sensitive to water contamination in organic normal-phase solvents, requiring the use of dry sample and mobile-phase solvents. Lastly, another limitation of cellulose-based columns is that they generally have lower plate numbers (less efficient) than protein-based columns. This characteristic often dictates that selectivity factors (α) be maximized for optimum separations.

The need for maximizing the analysis and isolation of chiral compounds led us to study ways that α-values for cellulose-based columns could be rapidly optimized. Optimization usually involves selecting a mobile phase and other operating conditions that produce the highest α-value, while maintaining good column efficiency. As a background for our work, the studies of Okamoto et al. [10], Wainer et al. [11] and Shibata et al. [4] were useful in promoting insight for expanding the range of typically-used solvents for the cellulose-based columns. Traditionally, cellulose-based column manufacturers recommend that protic (proton donating) modifiers are to be used with non-polar mobile-phase carriers (e.g., hexane) to resolve enantiomers [9,12]. Precautionary statements furnished with columns warn of adverse effects on performance and stability if there are deviations from suggested mobile-phase compositions.

This study has identified that certain non-protic modifiers often can improve chiral separations. At times, an aprotic modifier can produce a useful separation that is not possible with traditional protic modifiers. Use of polar modifiers other than alcohols greatly expands the applicability of the cellulose-based columns, without apparent column stability difficulties.

Three main objectives of this study were defined. First, data were needed to identify and quantify the effects of using aprotic modifiers with cellulose-based chiral columns. Second, there was an internal need to resolve and preparatively isolate a proprietary racemic aryl-oxy-propionate mixture using a derivatized cellulosic column. Finally, a simple optimization strategy for using cellulose-based columns was desired so that future analytical and preparative separations could be quickly and effectively completed.

2. Experimental

Chiralcel OD and OJ columns (50 × 4.6 mm I.D.), Chiralcel OC, OF and OG columns (250 ×

4.6 mm I.D.) and all HPLC-grade solvents were from J.T. Baker (Phillipsburg, NJ, USA). The HPLC apparatus consisted of a Model 400 pump (ABI Analytical, Ramsey, NJ, USA), a Model 7125 loop sample injector (Rheodyne, Cotati, CA, USA), and a variable-wavelength UV detector (ABI, Model 783G). Chromatographic data were acquired and analyzed with a Multichrome system (version 2.0, VG Laboratory Systems, Manchester, UK). Detector output also was monitored with a strip-chart recorder (Model BD-41, Kipp and Zonen, Delft, Netherlands).

Proprietary drug candidates, designated aryloxy propionate "ester", aminotetralin, aminoquinuclidine and aryl carbamate, were synthesized by ZENECA Pharmaceuticals (Wilmington, DE, USA). The general structural characteristics of these compounds are shown in Fig. 1. Stock solutions of 1 mg/ml for these compounds were prepared in ethanol or methanol, followed by dilutions with hexane to a concentration of 10 μg/ml. Injection volumes were 10 μl. At least duplicate injections were made with all solutions to verify that columns were equilibrated and retentions reproducible.

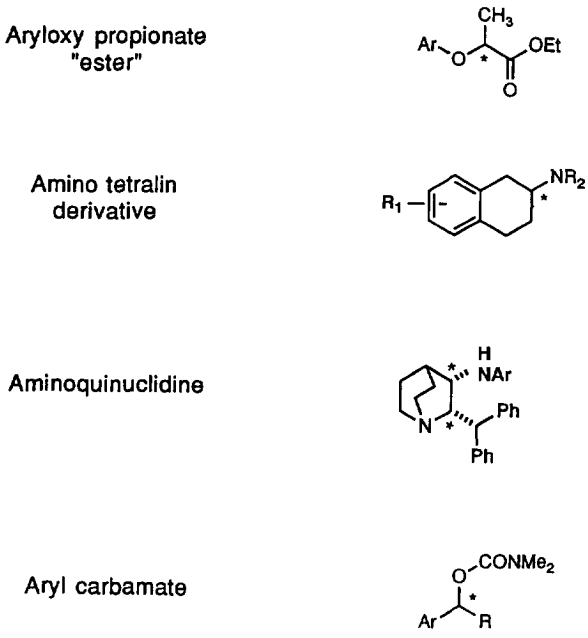

Fig. 1. Structures of compounds studied.

3. Results and discussion

For this study we chose to focus on the tris-3,5-dimethylphenylcarbamate column (Chiralcel OD), because of the large number of successful applications that have been successfully achieved with this stationary phase. Of 510 racemates tested, 229 (about 62%) were completely resolved with this column [13]. It has been indicated that 40% of the analytical sales and 70% of preparative isolations are performed with cellulose-based columns with this stationary phase [14]. However, other stationary phases are included in this study for comparison.

The mechanism for the enantioselectivity of carbamate–cellulose columns has been proposed by Okamoto and co-workers [4,13,15,16]. The suggested recognition processes with solutes include: (1) hydrogen bonding, (2) dipole–dipole interaction, (3) $\pi-\pi$ interactions, and (4) possible inclusion into chiral cavities [17]. Hydrogen-bonding plays a strong role in the selective chiral interaction process, and this interaction can occur at two potential sites of the carbamate functionality, as illustrated by the arrows in Fig. 2.

Hydrogen bonding is possible for both the solute and a protic (proton donating) modifier. This results in a competition between the solute and the protic solvent with the stationary phase for chiral recognition and separation resolution. For separations where hydrogen bonding pre-

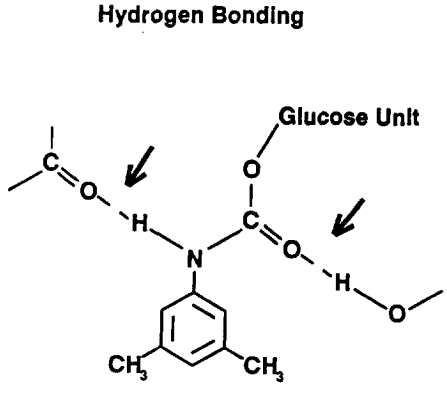

Fig. 2. Proposed mechanism for chiral recognition on cellulose–carbamate derivatives. Adapted from Ref. [10].

dominates, desired chiral interactions with solutes should then be facilitated by selecting a mobile-phase modifier that ineffectively competes by hydrogen bonding for chirally-active sites on the stationary phase. This postulation led to trials with aprotic solvents in the mobile phase to reduce hydrogen-bonding competition of the organic modifier with stationary-phase sites, compared to protic solvents.

Separations with the cellulose-based column packings have historically been performed with hexane carrier modified with various aliphatic alcohols. An interesting pattern is found for the resolution of the chiral "ester" compound (Fig. 1) on the popular Chiralcel OD column. Fig. 3A shows the effect of methanol, ethanol and 1-propanol modifiers in hexane. With this column, no resolution occurs with methanol and ethanol modifiers, but 1-propanol produces a resolution of about 0.9.

Fig. 3B shows that somewhat poorer resolution was obtained with 2-propanol as the modifier. But, as the size of the alcohol increased, resolution also increased, with *tert.*-butanol modifier giving the highest α-value. This effect may be based on the reduced capability of larger alcohols to compete for hydrogen-bonding sites because of steric limitations. (A higher concentration of this alcohol was required to maintain similar retention; a modest resolution increase may have occurred as a result of increased k' values). In spite of this higher α-value, resolution was somewhat lower for *tert.*-butanol compared to iso-butanol. This probably was the result of poorer column efficiency (plate number $N = 524$ for *tert.*-butanol; $N = 727$ for iso-butanol), which may be caused by less favorable kinetics with the solvated stationary phase. Highest column efficiency for the C_4 alcohols was found for 1-butanol, even though α (and resolution) values were lower. These results suggest that mobile-phase modifiers with reduced tendency to interact by hydrogen bonding with the modified cellulose stationary phase can provide higher enantiomeric resolution for compounds that also compete for these hydrogen-bonding, chirally-active sites.

An attempt was made to correlate some basic characteristic of alcohol modifiers to enantiomeric selectivity. No useful correlation was found with the solvent polarity parameter described by Snyder [18]. However, a reasonable correlation was obtained using the solvent strength parameter, ϵ^0, also described by Snyder for use in liquid–solid chromatography [19], as illustrated by the data in Fig. 4. This result supports a mechanism involving the competition of the solute and the modifying polar solvent for hydrogen bonding sites on the stationary phase. The interaction may be somewhat similar to the site-competition process now accepted as the mechanism for liquid–solid or adsorption chromatography [19].

Utilizing aprotic solvents as mobile-phase modifiers apparently reduces the competition of the solvent for the hydrogen-bonding sites on the chiral stationary phase, increasing the possibility of the solute to interact at these sites. The effect of aprotic mobile-phase modifiers with the Chiralcel OD column is shown in Fig. 5. Fig. 5A shows that acetonitrile and tetrahydrofuran produce no improvement over results with the protic modifiers in Fig. 3. This suggests that both acetonitrile and tetrahydrofuran are strongly held to hydrogen-bonding sites of the chiral stationary phase. However, as shown in Fig. 5A, the aprotic methyl-*tert.*-butyl ether modifier (MTBE) produced a resolution of 1.70. (Note that 25% of this solvent modifier was required to maintain comparable retention). This resolution of 1.70 significantly exceeds the best previously found with the alcohol modifiers ($R_s = 1.30$ for iso-butanol in Fig. 3B). Fig. 5B shows that aprotic methylene chloride and ethyl acetate modifiers both provide resolutions of > 1.2 for the chiral "ester". Methylene chloride exhibited the most favorable effect on column efficiency, producing the largest plate number of any solvent tested with this solute.

Based on use-recommendations by the manufacturer, we were concerned about the stability of the Chiracel OD column when employing aprotic solvent modifiers. Fig. 6 shows comparative chromatograms obtained with a fresh column and one with a history of about 400 sample injections, using a mobile phase of 25% methyl-

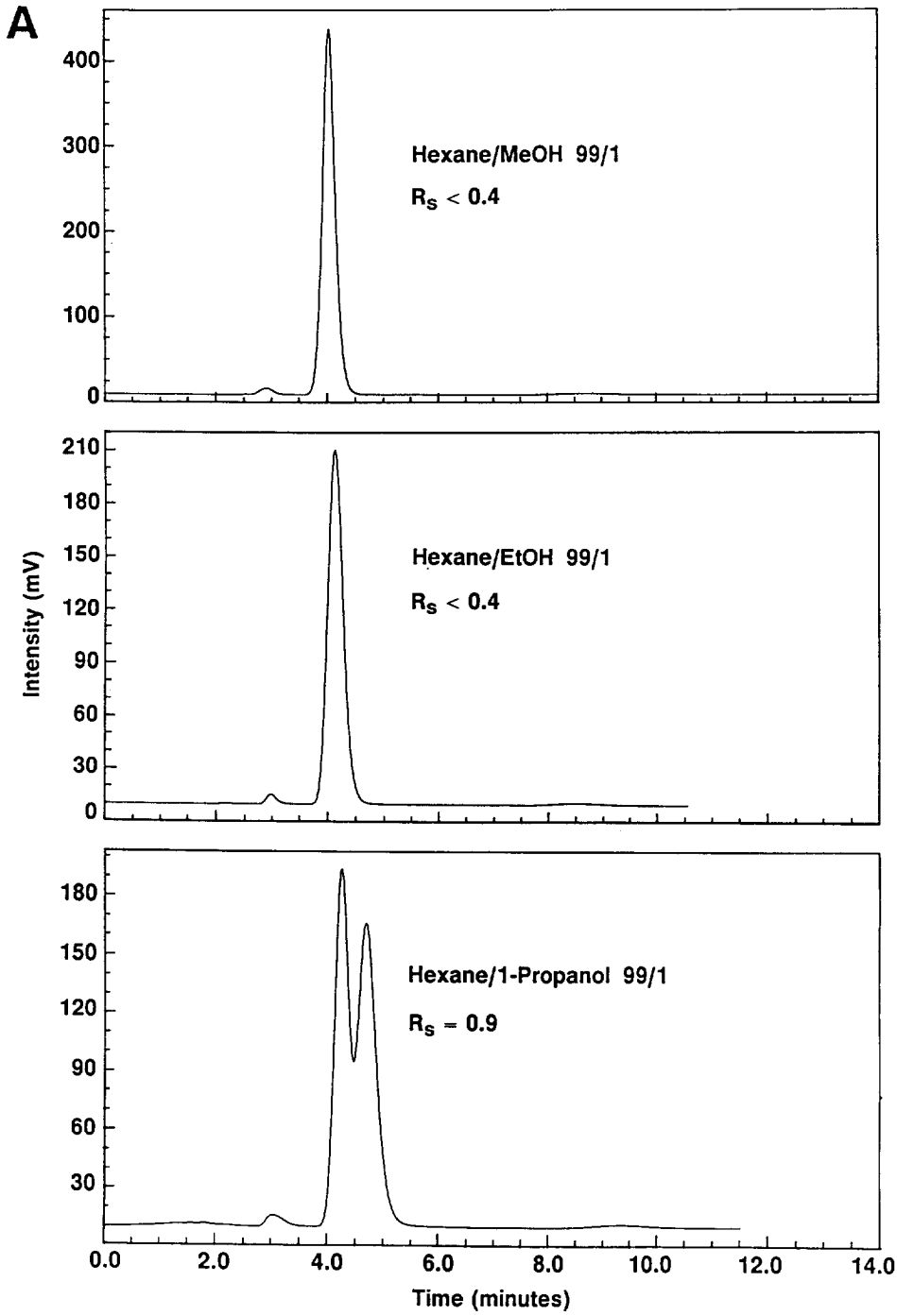

Fig. 3. Effect of protic mobile phase modifiers on resolution of chiral ester. Column, 50 × 4.6 mm I.D. Chiralcel OD; solute, "ester" in Fig. 2; flow-rate, 0.5 ml/min; temperature, ambient; UV detector, 230 nm. (A) Methanol, ethanol, 1-propanol modifiers; (B) 2-propanol, 1-butanol, isobutanol, *tert.*-butanol modifiers.

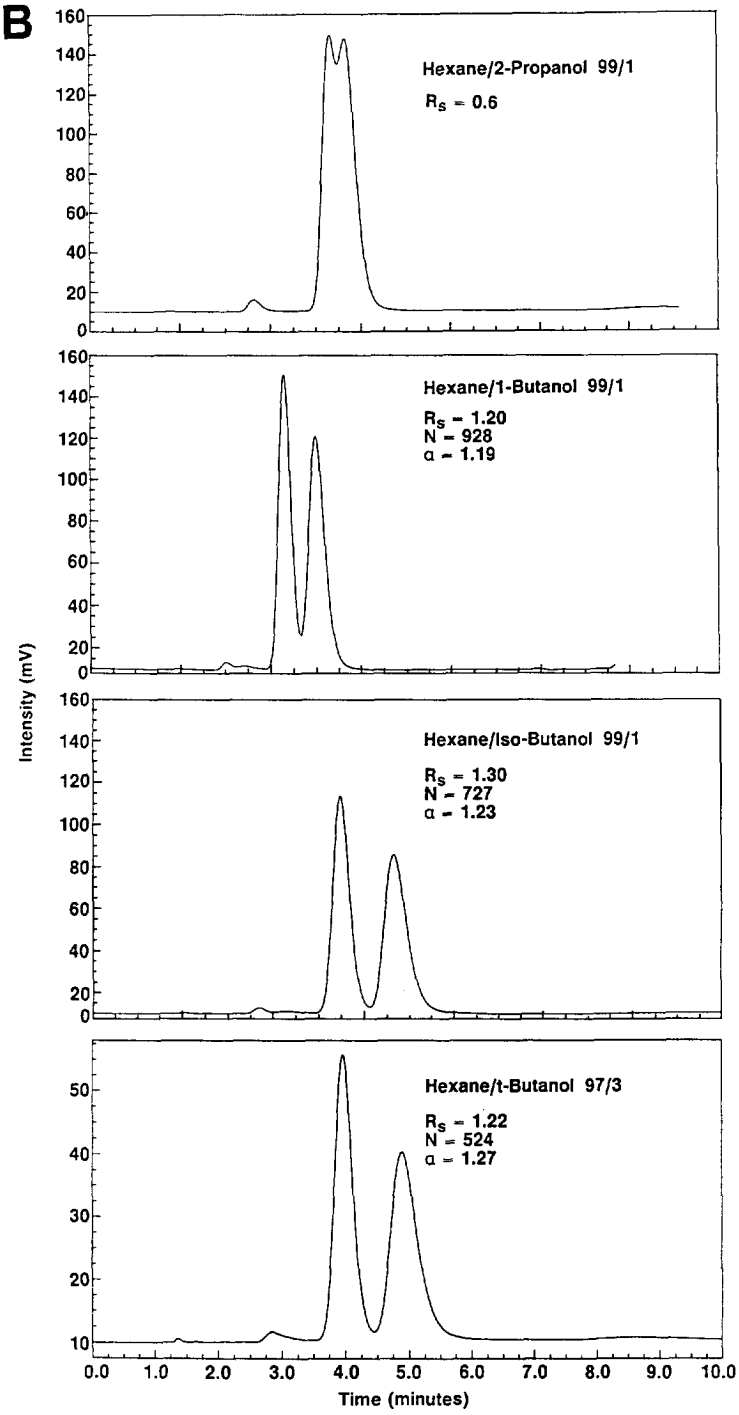

Fig. 3. (*continued*)

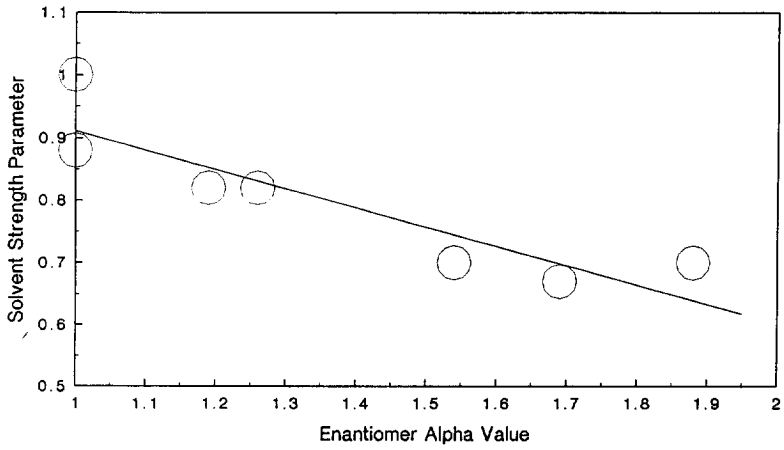

Fig. 4. Correlation of solvent strength ϵ^0 with selectivity α-values. Conditions of Fig. 3.

tert.-butyl ether–hexane. Essentially equivalent retention and resolution for the two columns suggest satisfactory column stability in such mobile phases. In obtaining the data for this study, we used compositions up to 50% methyl-*tert.*-butyl ether, 30% tetrahydrofuran, 10% methylene chloride and 10% ethyl acetate with the Chiracel OD column without column stability problems.

The short (5-cm) columns were used for method development because of convenience and cost. Comparative chromatograms for the racemic ester with 5- and 25-cm columns are given in Fig. 7. Curiously, with this mobile phase of 25% methyl-*tert.*-butyl ether in hexane, the expected five-fold increase in plate number was not obtained with the 25-cm column. The plate height for the 5-cm column was 0.00625 cm at a flow-rate of 0.5 ml/min, compared to 0.0202 for the 25-cm column at a flow-rate of 1.0 ml/min. We speculate that the shorter 5-cm column may have contained smaller particles, or was more efficiently packed.

A brief study was made on the batch-to-batch reproducibility for the 5-cm columns. Three different 5-cm columns of Chiracel OD (one used for these studies, two fresh columns) showed resolutions of 1.25 to 1.70 for the same enantiomers. No changes occurred in resolution for the initial column used in this study ($R_s = 1.70$ for these enantiomers). This finding suggests that, while these short columns are quite useful for research and certain method studies, they may not be sufficiently reproducible for regulatory (Good Laboratory/Manufacturing Procedures) operations.

Table 1 summarizes results of using different mobile phases with the Chiracel OD column in the resolution of enantiomers for the "ester" type drug. Again, the addition of acetonitrile to the ethanol–hexane mobile phase affected α-values very little. However, with aprotic acetonitrile modifier, column plate number increased dramatically, resulting in a significant increase in enantiomer resolution. We speculate that this higher plate number may result in better kinetics (mass transfer) with the lower-viscosity acetonitrile-modified mobile phase. This effect is well known in the reversed-phase separation of achiral compounds, where acetonitrile as mobile-phase modifier (instead of alcohols) decreases column back pressure and increases column plate number [20]. As shown in Table 1, adding methyl-*tert.*-butyl ether or ethyl acetate to the ethanol–hexane mobile phase had little effect on chiral resolution.

As shown by the Chiralcel OD data in Fig. 8 for the racemic aryl carbamate, α-values and separation resolution were highest when higher alcohols and methyl-*tert.*-butyl ether were used as mobile-phase modifiers. Interestingly, plate number was lowest and α value highest with

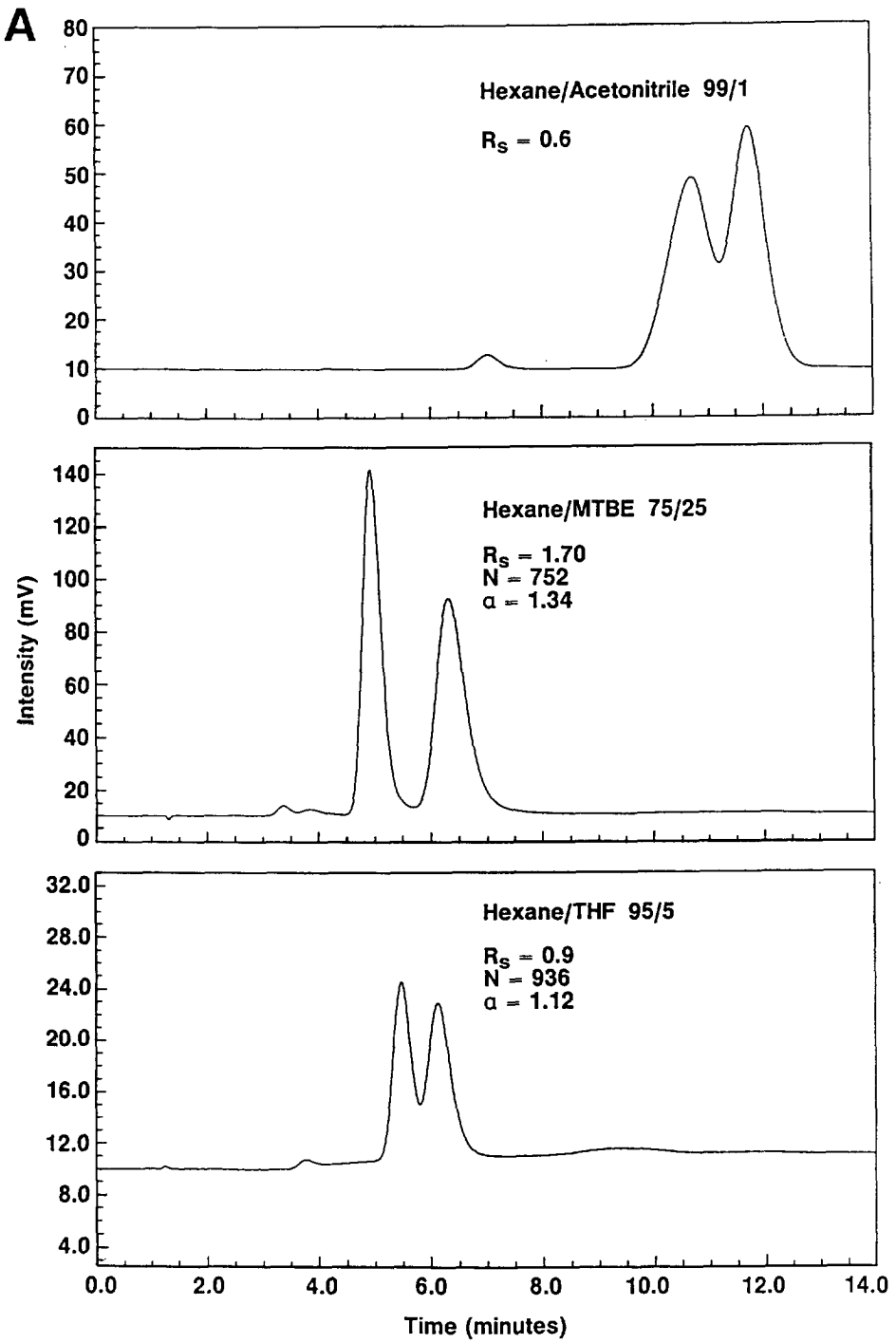

Fig. 5. Effects of aprotic modifiers on resolution of chiral ester. Conditions as for Fig. 3. (A) Acetonitrile, methyl-*tert.*-butyl ether (MTBE) and tetrahydrofuran (THF) modifiers; (B) methylene chloride ($MeCl_2$) and ethyl acetate (EtAc) modifiers.

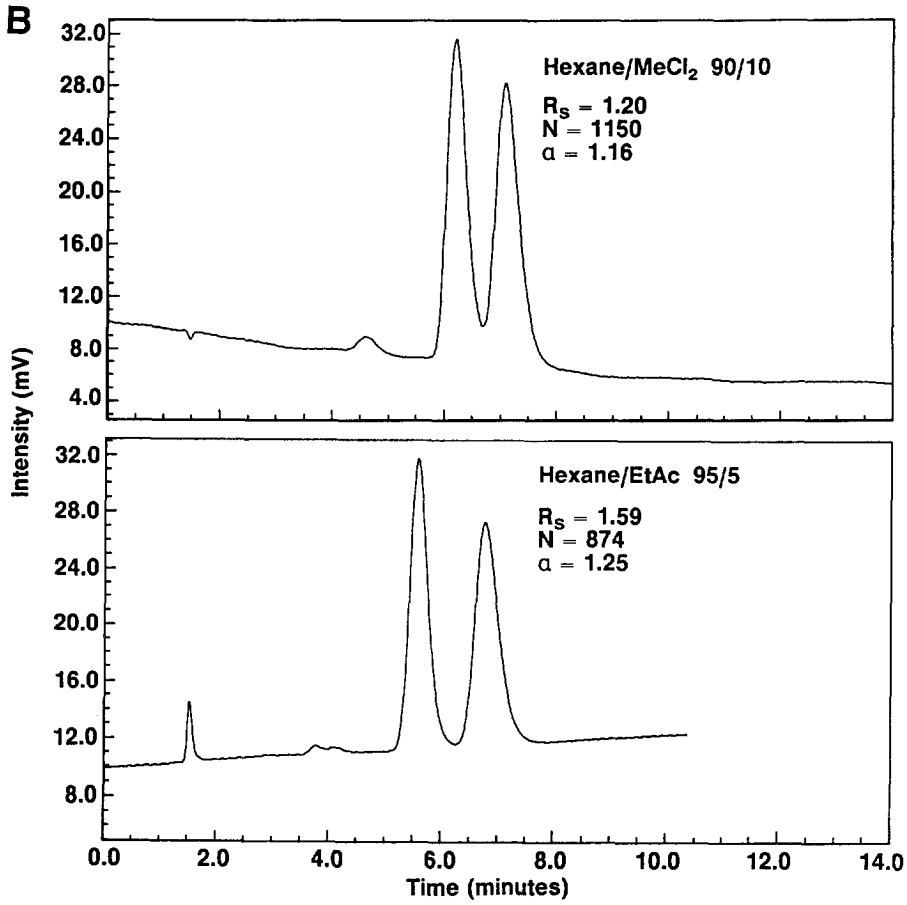

Fig. 5. (*continued*)

methyl-*tert*.-butyl ether as the modifier. These results are somewhat similar to those for the aryloxy propionate "ester" previously discussed.

However, aprotic mobile-phase modifiers are not required with the Chiracel OD column for adequate resolution of all racemates. For example, Table 2 gives retention data for three mobile phases tested with the amino-tetralin drug (Fig. 1). In this case, adding protic solvents such as acetonitrile and MTBE to the mobile phase improved the resolution of these enantiomers very little. Best separation was obtained with methanol, which has the strongest capability to interact with the chirally-active sites on the stationary phase by hydrogen bonding. The results in Tables 1 and 2 suggest, therefore, that hydrogen-bonding interactions are not a dominating feature of chiral interactions that affect enantiomeric selectivity for all drugs. Note that where the ethanol concentration exceeds 5% for proper elution, direct replacement with an aprotic solvent usually is not feasible.

A convenient method was sought for adjusting solvent strength for the Chiracel OD column when replacing protic with aprotic solvents for selectivity enhancement. As noted in Fig. 4, Snyder's solvent strength parameter, ϵ^0, seems to relate to α for the cellulose-based columns, suggesting that stronger solvents more effectively compete for chirally-active sites on the stationary phase than the solute. Based on limited data, it appears that ϵ^0-values for the amino bonded phase given by Snyder and Schunk [21] may be useful in relating the strength of one modifier to another. For example, the chiral ester drug

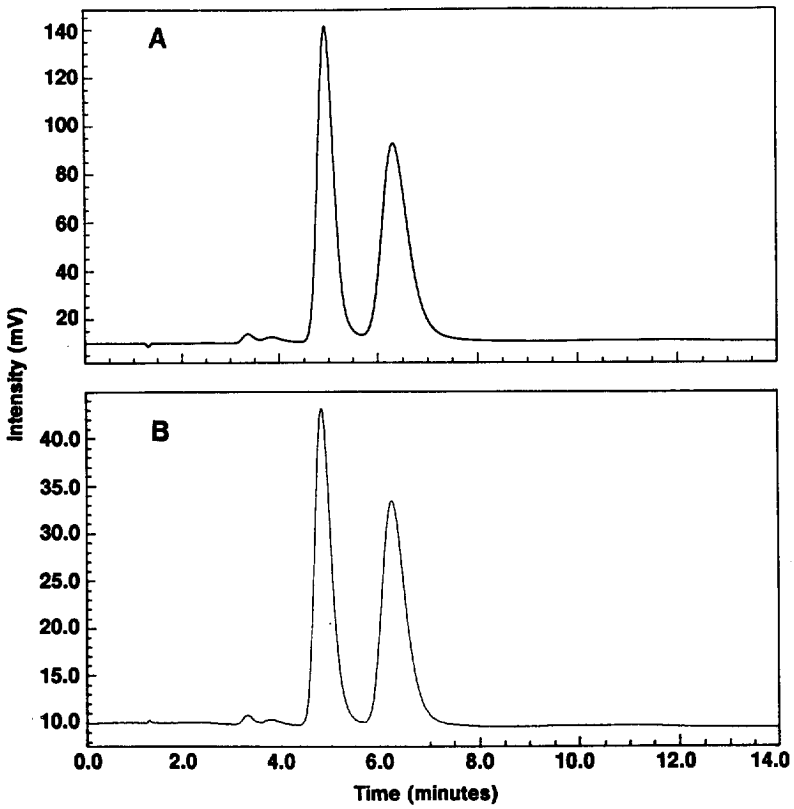

Fig. 6. Stability of cellulose-based chiral column in aprotic solvent-modified mobile phase. Column, 50 × 4.6 mm I.D. Chiralcel OD; solute, "ester" in Fig. 1; mobile phase, methyl-*tert.*-butyl ether–hexane (25:75, v/v); flow-rate, 0.5 ml/min; temperature, ambient; UV detector, 230 nm. (A) Fresh column; (B) after 400 sample injections.

shows about the same retention (k'-values) with 25% MTBE, 10% methylene chloride and 1% ethanol in hexane. According to Ref. [20], 28% MTBE, 11% methylene chloride and 1% ethanol in hexane all have a solvent strength parameter value of 0.04. These results suggest the possibility of using solvent strength values to characterize retention with the Chiralcel OD column, but more work in the area is needed.

What is the effect of aprotic modifiers on enantiomer resolution that can be expected for other carbamate stationary phases? As shown in Figs. 9A and 9B, no resolution was found for the enantiomers of the aryloxy propionate "ester" with Chiracel OC and OG columns when alcohol (protic) modifiers were used. Hexane modified with MTBE (aprotic) produced partial resolution with the Chiralcel OC column (Fig. 9A) and excellent resolution with the Chiracel OG column (Fig. 9B). For this drug, the Chiracel OF column produced the best results with methyl-*tert.*-butyl ether modifier (Fig. 9C).

The effect of various protic modifiers on the enantioselectivity of the Chiralcel OJ column is demonstrated in Fig. 10 for the chiral "ester" compound (Fig. 1). No separation of enantiomers occurred with 5% methanol as the modifier when using a 5-cm column (Fig. 10A). Ethanol and propanol at the same concentration produced resolutions of about 0.8 and 0.7, respectively. All of these solvents have strong hydrogen-bonding capabilities, with methanol the most polar and the most basic of the three. One could speculate that the sample has the most difficulty interacting with this chiral cellulose stationary phase in the presence of tightly-held methanol.

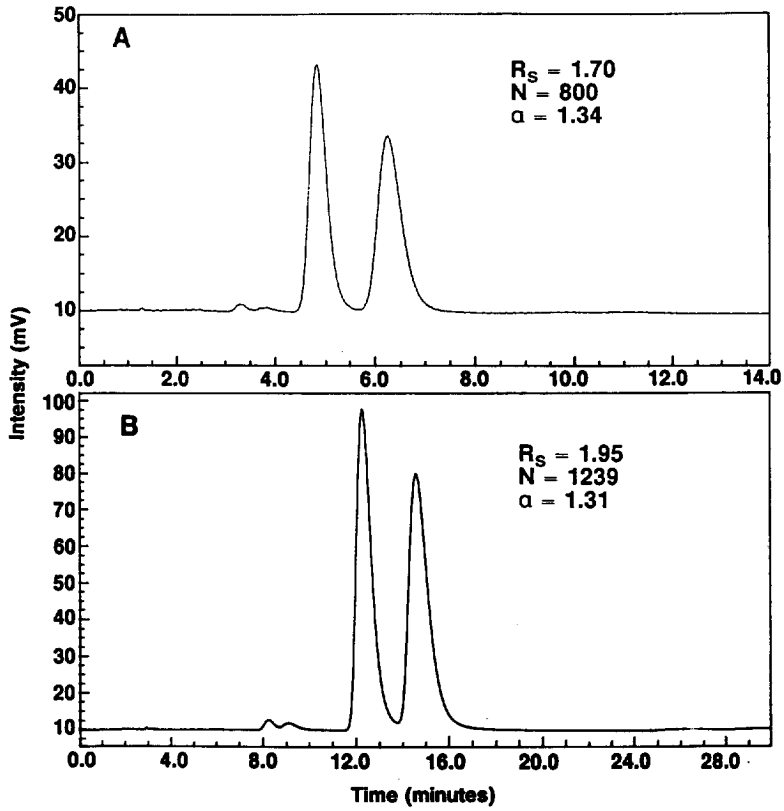

Fig. 7. Comparison of column configurations. Conditions of Fig. 6, except: (A) 250 × 4.6 mm I.D. column; flow-rate, 1.0 ml/min; (B) 50 × 4.6 mm I.D. column; flow-rate, 0.5 ml/min.

The desired chiral interaction occurs with ethanol as modifier, even though this is a more polar and more basic solvent than 1-propanol. The interactive process likely involves, but is not exclusively dependent on, hydrogen bonding. Note that increasing the steric bulk of the alcohol modifier or substituting the aprotic methyl-*tert.*-butyl ether solvent did not improve the resolution of the "ester" enantiomers on Chiracel OJ (Fig. 10B).

Based on the results of this study, a simple strategy was devised for optimizing chiral separations on the Chiralcel OD column. A synopsis of this strategy is given in Fig. 11. Ethanol-

Table 1
Summary of chromatographic data on candidate "ester" drug with Chiralcel OD column

Mobile phase (v/v)	k_1'	k_2'	Selectivity, α	Resolution, R_s	Plate number[a]
Hexane–ethanol (99:1)	2.63	3.23	1.23	1.18	1348
Hexane–ethanol–acetonitrile (99:1:1)	1.90	2.20	1.15	1.51	3475
Hexane–ethanol–methyl-*tert.*-butyl ether (99:1:1)	2.90	3.63	1.25	1.30	1189
Hexane–ethanol–ethyl acetate (99:1:1)	2.37	2.83	1.19	1.18	1575

[a] Plate number of second enantiomer.

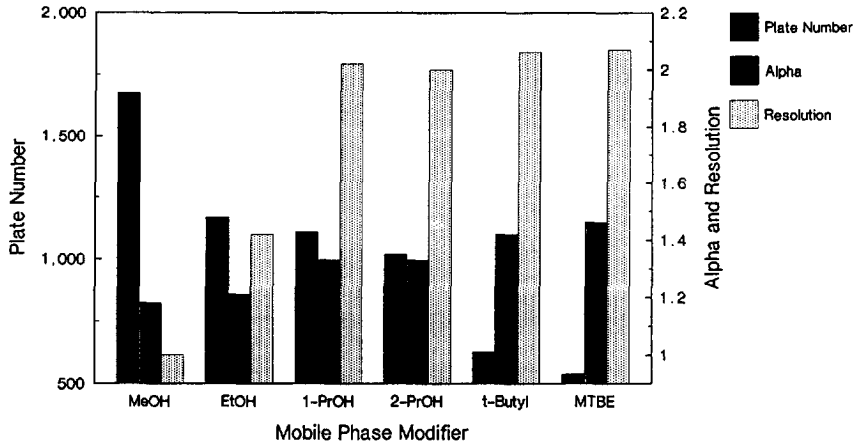

Fig. 8. Effect of mobile phase modifier on chromatography of carbamate drug. Column: 50 × 4.6 mm I.D. Chiralcel OD; solute, carbamate drug of Fig. 1; mobile phases, 5% (v/v) modifier, except 50% methyl-*tert.*-butyl ether; flow-rate, 0.5 ml/min; temperature, ambient; UV detector, 220 nm.

modified hexane is recommended as the starting mobile phase. The suggested initial ethanol level (10%) should be adjusted to obtained the desired retention ($k' = 1–10$) for all compounds. If inadequate resolution is obtained, bulkier (larger) alcohols are substituted as modifiers, again keeping retention in the desired range by adjusting alcohol concentration. If inadequate resolution again is obtained, an aprotic solvent modifier such as methyl-*tert.*-butyl ether, acetonitrile, or methylene chloride should be substituted. If no or partial resolution is obtained, column temperature or column type must be changed.

4. Conclusions

Aprotic solvent modifiers are useful for resolving drug enantiomers on derivatized cellulose carbamate HPLC stationary phases. Optimization of chiral separations on the Chiralcel OD column can usually be achieved with a limited number of mobile-phase combinations and short (5-cm) columns. Normally-used alcohol modifiers often can be replaced by certain aprotic solvents for superior separations. Based on these results, improvement in enantiomer resolution with aprotic solvents largely occurs when hydrogen bonding is the major source of chiral inter-

Table 2
Summary of chromatographic data on candidate amino-tetralin drug on Chiralcel OD

Mobile phase (v/v)	k_1'	k_2'	Selectivity, α	Resolution, R_s	Plate number[a]
Hexane–ethanol (98:2)	8.98	9.75	1.09	0.6	N/A[b]
Hexane–ethanol–acetonitrile (98:2:2)	6.74	7.42	1.10	0.7	N/A
Hexane–methanol (98:2)	9.53	11.27	1.18	2.37	1647

[a] Plate number of second enantiomer.
[b] Not available; peaks badly overlap.

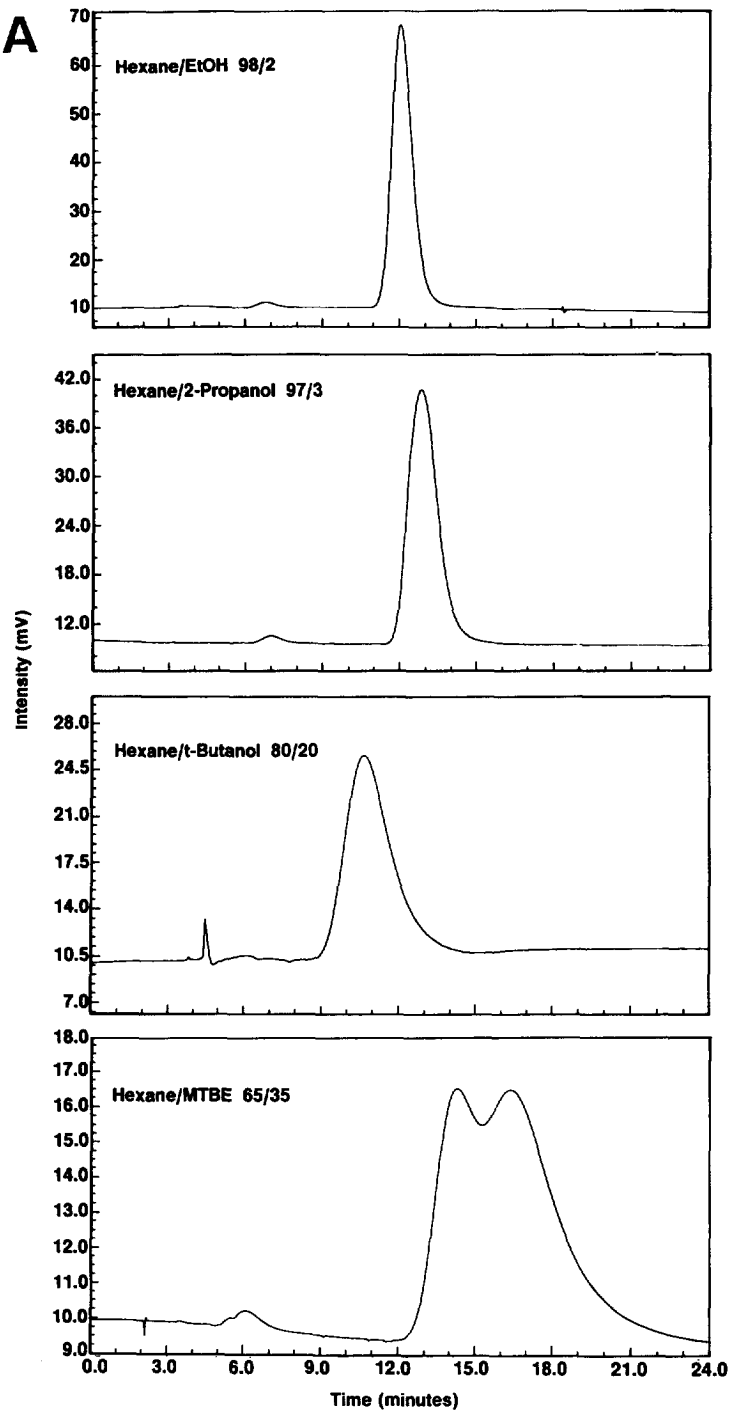

Fig. 9. Effect of stationary phase functionality and organic solvents on the separation of chiral "ester" enantiomers. Columns: 250 × 4.6 mm I.D.; temperature, ambient; UV detector, 230 nm; mobile phases as shown; flow-rate, 1.0 ml/min. (A) Chiralcel OC; (B) Chiralcel OG; (C) Chiralcel OF.

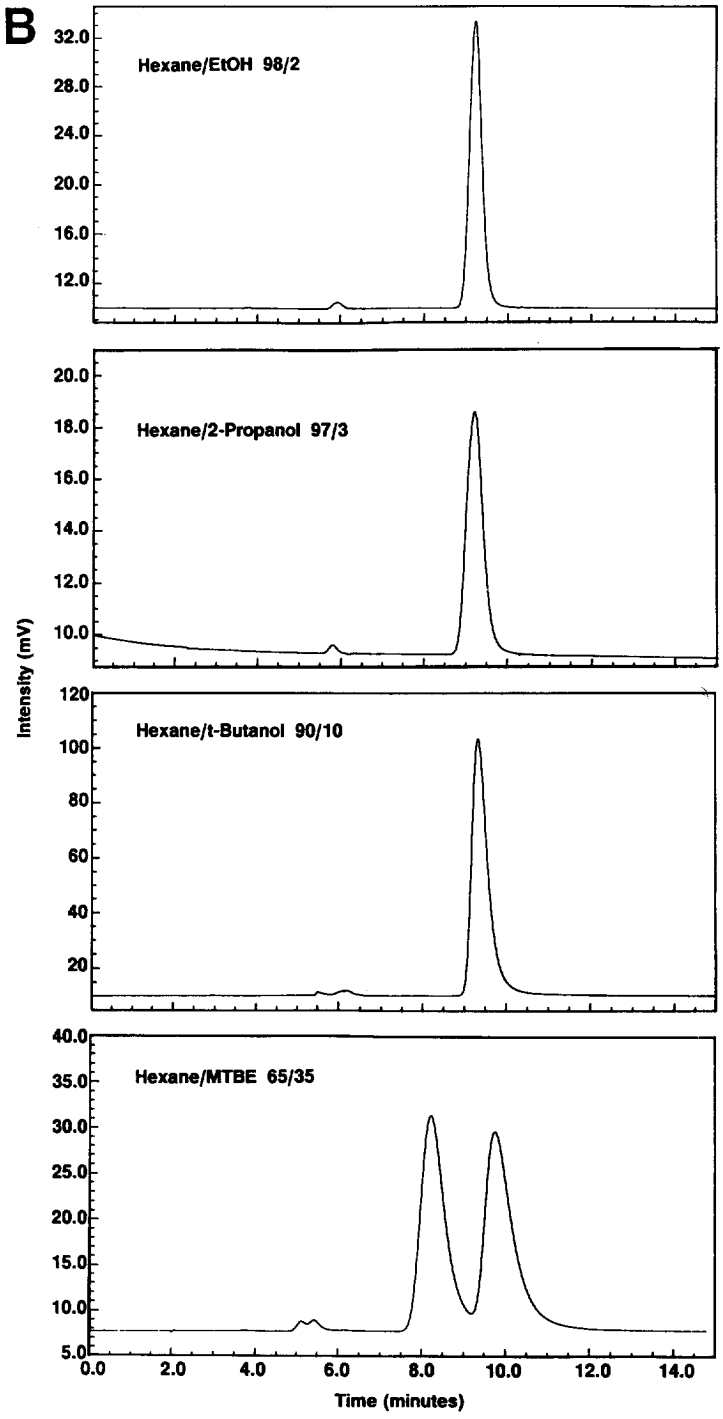

Fig. 9. (*continued*)

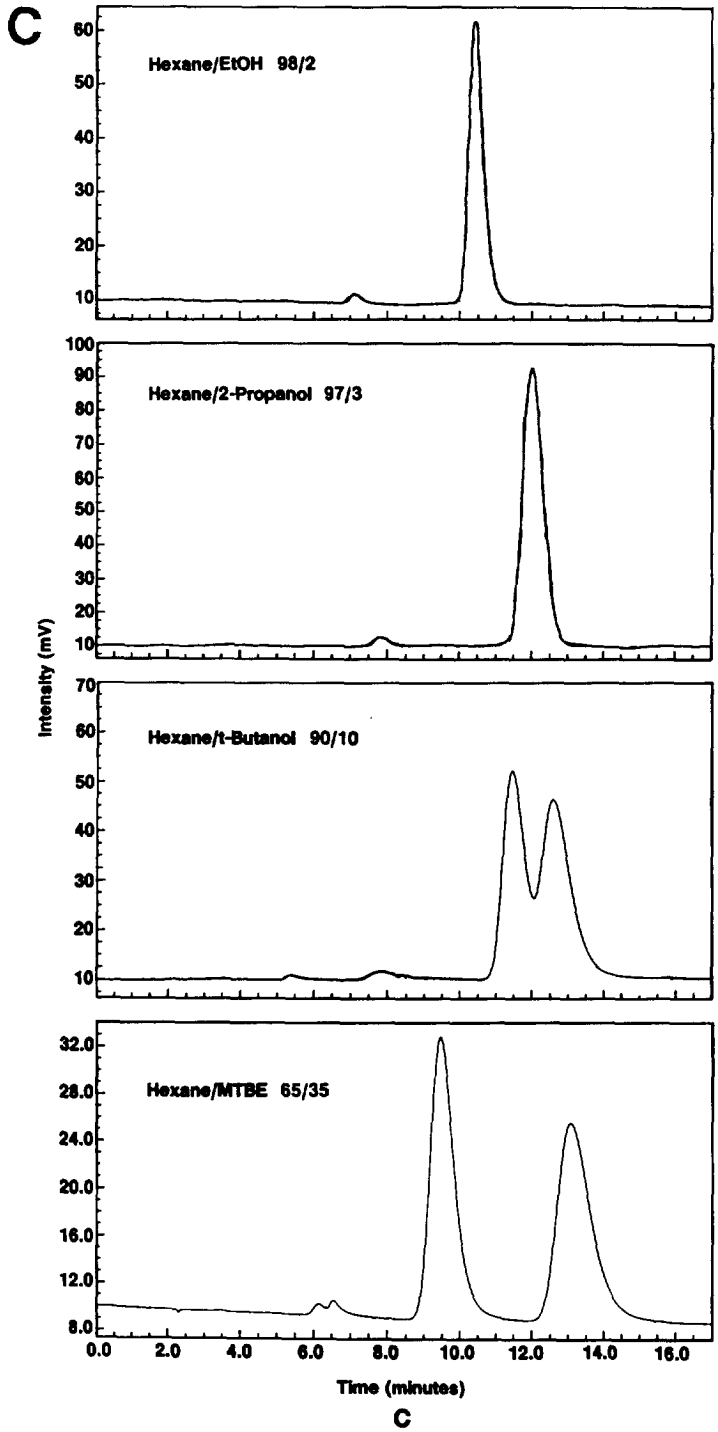

Fig. 9. (*continued*)

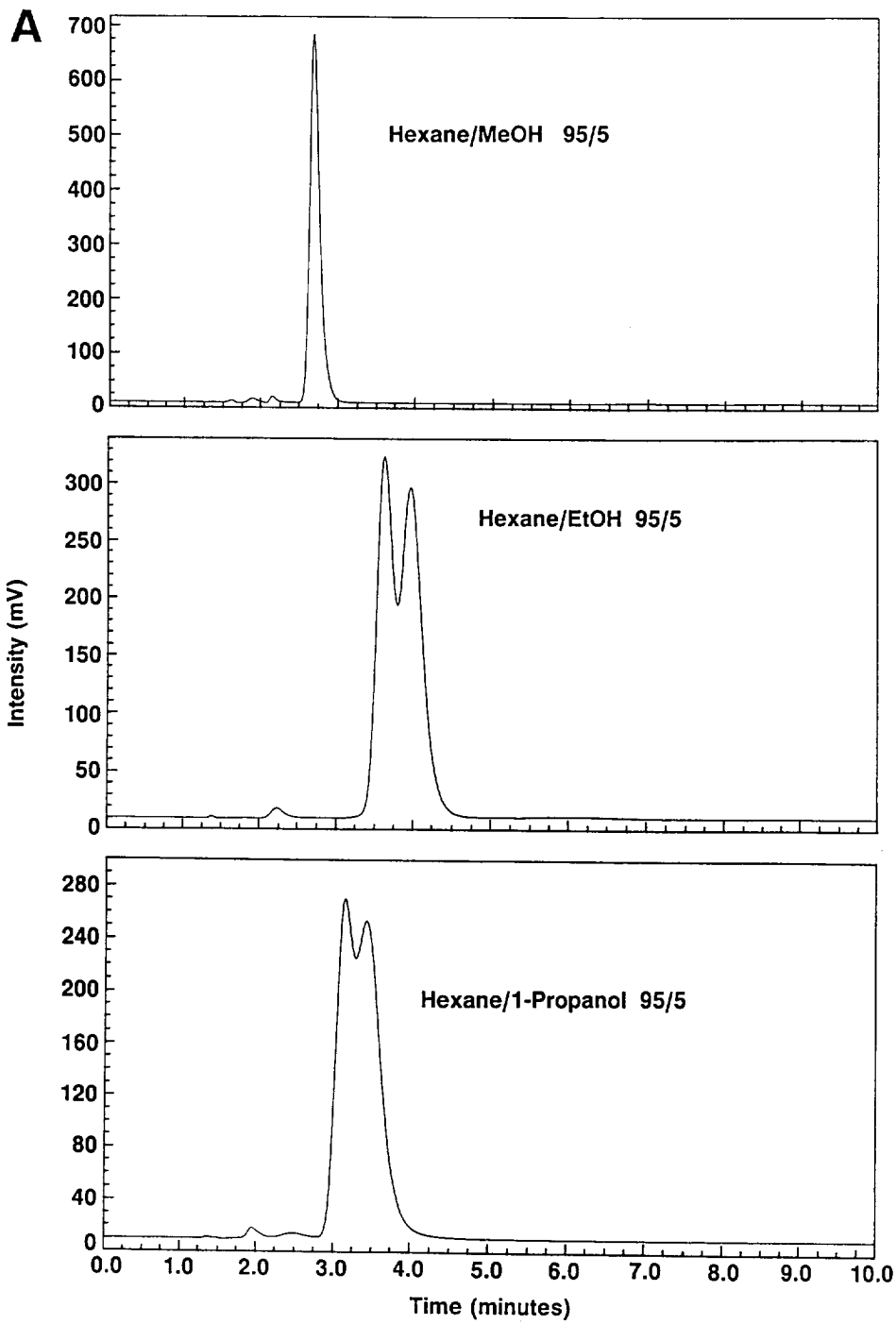

Fig. 10. Effect of protic modifiers on selectivity with the Chiralcel OJ column. Column: 50 × 4.6 mm I.D. Chiralcel OJ; solute, chiral "ester" of Fig. 1; flow-rate, 0.5 ml/min; temperature, ambient; UV detector, 230 nm. (A) Hexane–methanol, ethanol, 1-propanol; (B) hexane–2-propanol, *tert.*-butanol, methyl-*tert.*-butyl ether.