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Journal of Chromatography A, 855 (1999) 411–421

JOURNAL OF CHROMATOGRAPHY A

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Application and comparison of derivatized cellulose and amylose chiral stationary phases for the separation of enantiomers of pharmaceutical compounds by high-performance liquid chromatography

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Received 31 March 1999; received in revised form 11 June 1999; accepted 11 June 1999

Abstract

The direct high-performance liquid chromatographic separation of three pairs of structurally related enantiomers on derivatized cellulose and amylose chiral stationary phases (Chiralcel OD, Chiralpak AD and Chiralpak AS) was studied using hexane as the mobile phase with 2-propanol or ethanol as modifiers. The separation, retention and elution order of the enantiomers on the different columns using different alcohol modifiers were compared. The effect of structural variation of the solutes on their k' was noted. A reversal of elution order of one enantiomeric pair upon changing the mobile-phase modifier was observed. Chiralcel OD and Chiralpak AD columns provided different elution orders of the enantiomers, including a fourth pair of enantiomers that were not structurally related to the other three pairs. © 1999 Published by Elsevier Science B.V. All rights reserved.

Keywords: Chiral stationary phases, LC; Mobile-phase composition; Cellulose stationary phases; Amylose stationary phases; Enantiomer separation; Pharmaceutical analysis; Morpholines

1. Introduction

Enantiomers of pharmaceutical compounds may display quite different pharmacological behaviors [1]. Therefore, the development of analytical methods that can separate and quantify the enantiomers plays a very important role in the drug development process. The most popular technique used for the separation and quantification of the enantiomers is high-performance liquid chromatography (HPLC).

In the development of a HPLC method, it is usually desirable to use a chiral stationary phase (CSP) to directly separate the enantiomers because of the simplicity and ease of operation related to this approach. There are various types of CSPs available. Several books [2–5], review articles [6,7] and research papers [8,9] describe the basis of separation on different columns and suggest columns that are best suited to certain types of analytes. Among the various CSPs, cellulose- and amylose-based CSPs have been proved to be quite versatile. A wide variety of enantiomeric compounds, including chiral aromatic alcohols [10], enantiomeric amides [11,12], pyriproxyfen [13], amino alcohols [14], diol [15],

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0021-9673/99/$ – see front matter
PII: S0021-9673(99)00733-5

β-blockers [16–18], racemic carboxylic acid [19] and other miscellaneous compounds [20–24], have been separated on these CSPs. A series of excellent review papers on these CSPs is available [21–24]. It was noted that the tris(phenylcarbamate) derivatives of cellulose and amylose are particularly effective.

In this paper, the separation, retention and elution order of four pairs of enantiomers were studied on three cellulose- or amylose-based CSPs, i.e., cellulose tris(3,5-dimethylphenylcarbamate) (Chiralcel OD), amylose tris(3,5-dimethylphenylcarbamate) (Chiralpak AD) and amylose tris[(*S*)-1-phenylethylcarbamate] (Chiralpak AS). Hexane containing an alcohol modifier (2-propanol or ethanol) was used as the mobile phase. The results obtained on different columns under various mobile-phase conditions were compared. The effect of structural variation of the solutes on their retention factors (k') was noted.

Very often, in addition to achieving the separation of the enantiomers, it is also desirable to elute the minor enantiomer before the major one, to avoid possible interference caused by the tail of the major enantiomer, especially when the separation is marginal. Therefore, looking for different ways to achieve a reversal of elution order is of much interest to researchers. In this study, it was found that the elution order of one enantiomeric pair was reversed by changing the alcohol modifier in the mobile phase. In addition, the elution order of all of the enantiomeric pairs was reversed by changing the stationary phase from the amylose-based Chiralpak AD to its cellulose-based counterpart, Chiralcel OD. This observation could be used as a guide to future method development.

2. Experimental

2.1. Instrumentation

The chromatography was performed with a Hewlett-Packard 1100 HPLC system equipped with a photodiode array detector (Wilmington, DE, USA). The stainless steel columns (25 cm×4.6 mm) packed with Chiralcel OD [cellulose tris(3,5-dimethylphenylcarbamate) coated on silica gel], Chiralpak AD [amylose tris(3,5-dimethylphenylcarbamate) coated on silica gel] and Chiralpak AS {amylose tris[(*S*)-1-phenylethylcarbamate] coated on silica gel} were purchased from Chiral Technologies (Exton, PA, USA). Chromatograms were acquired and processed using a PE Nelson data system equipped with Access*Chrom software (version 1.9.5) (PE Nelson, Cupertino, CA, USA).

2.2. Materials

The HPLC-grade hexane (catalog no. H302-4) and 2-propanol (IPA) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). The 200 proof, dehydrated ethanol was purchased from Quantum Chemical (Newark, NJ, USA). Authentic samples of 3-(*S*)-(4-fluoro)phenyl-4-benzyl-2-morpholinone (Compound A), 3-(*R*)-(4-fluoro)phenyl-4-benzyl-2-morpholinone (Compound A′), 2-(*R*)-[3,5-bis(trifluoromethyl)benzoyloxy] - 3(*S*) - (4 - fluoro)phenyl-4-benzylmorpholine (Compound B), 2-(*S*)-[3,5-bis-(trifluoromethyl)benzoyloxy]-3(*R*)-(4 - fluoro)phenyl-4-benzylmorpholine (Compound B′), 2-(*R*)-[1-(*R*)-3, 5 - bis (trifluoromethyl) phenylethoxy] - 3 (*S*) - (4 - fluoro)phenyl-4-(3-oxo-1,2,4-triazol-5-yl)methylmorpholine (Compound C), 2-(*S*)-[1-(*S*)-3,5-bis(trifluoromethyl)phenylethoxy] - 3(*R*) - (4 - fluoro)phenyl-4-(3-oxo-1,2,4-triazol-5-yl)methylmorpholine (Compound C′), 4-(*S*)-6-chloro-4-cyclopropyl-3,4-dihydro-4 - [2 - (2 - pyridyl)ethyn - 1 - yl]quinazolin - 2(1*H*) - one (Compound D) and 4-(*R*)-6-chloro-4-cyclopropyl-3,4-dihydro - 4 - [2 - (2 - pyridyl)ethyn-1-yl]quinazolin-2(1*H*)-one (Compound D′) were provided by the Process Research and Development Department of Merck Research Labs. (Rahway, NJ, USA). The syntheses of Compounds A, B and C were described in Ref. [25]. Compounds A′, B′ and C′ can be prepared using methods similar to those described in Ref. [25]. Ref. [26] described the syntheses of Compounds D and D′. The methods used to determine the absolute configurations of the chiral centers present in these compounds are beyond the scope of this paper and will be published elsewhere.

2.3. Chromatographic conditions

The mobile phase consisted of HPLC-grade hexane and an alcohol modifier (IPA or ethanol). The flow-rate was 0.5 or 1.0 ml/min. The column was at room temperature (~22°C). UV detection was per-

formed at either 220 or 260 nm. The retention factor, k', was determined as $k' = (t_R - t_0)/t_0$. The t_0 was determined by injecting hexane, which was a weaker solvent than the IPA–hexane or ethanol–hexane mixtures, and noting the time of appearance of the peak due to hexane [27].

2.4. Elution order of enantiomers

In the separation of each enantiomeric pair, the elution order of the enantiomers was determined using an arbitrary mixture of the pure enantiomers. In the mixture, the level of the desired enantiomer was elevated.

3. Results and discussion

The structures of the three pairs of structurally related enantiomers (Compounds A and A′, B and B′, C and C′) are shown in Fig. 1. Compounds A, B and C are the synthetically desired (major) enantiomers and Compounds A′, B′ and C′ are the undesired (minor) enantiomers. The three CSPs used in our study were cellulose- or amylose carbamate derivatives coated on silica gel. The structures of the derivatized subunits of the CSPs can be found in Refs. [21–23].

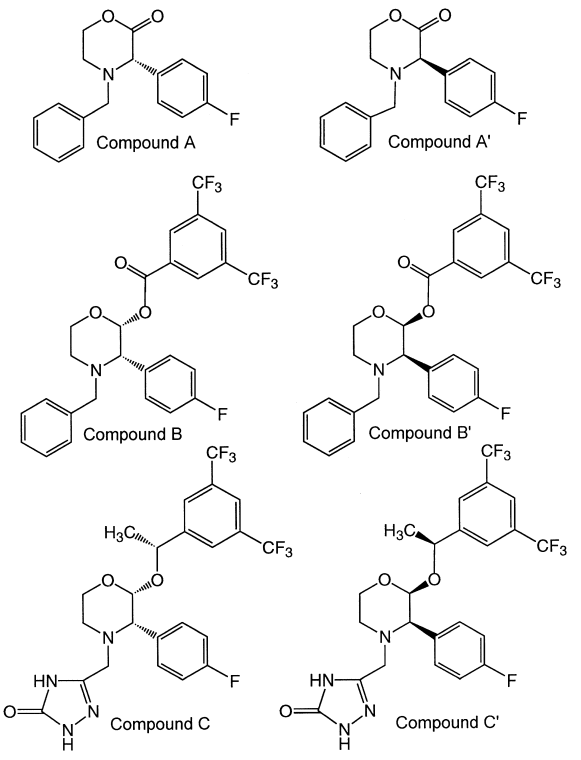

Fig. 1. Structures of the three pairs of structurally related compounds.

It has been assumed that the separation of enantiomers on the cellulose- and amylose-based CSPs was due to the formation of solute–CSP complexes between the enantiomers and the chiral cavities in the higher order structures of the CSPs [10,21,23]. In the CSPs with carbamate derivatives, such as the CSPs used in our study, the binding of the solutes to the CSPs was achieved through interactions between the solutes and the polar carbamate groups on the CSPs [21,23,24]. The carbamate groups on the CSP can interact with solutes through hydrogen bonding using the C=O and NH groups and through dipole–dipole interactions using the C=O moiety. In the cases of Compounds A and B, the C=O group on the solutes and the NH group on the stationary phases could form hydrogen bonding. In the case of Compound C, the NH and C=O groups on the solute could form hydrogen bonding with the C=O and NH groups on the CSPs. Dipole–dipole interactions could also occur between the C=O groups on all of the solutes and the C=O group on the stationary phases. Wainer et al. [10] have reported that the solute–CSP complex, formed between a solute having aromatic functionalities and cellulose-based CSP, can be stabilized by insertion of the aromatic portion of the solute into the chiral cavity. In our case, this type of stabilization interaction might also occur due to the presence of the aromatic functionalities on the solutes. Chiral discrimination between the enantiomers was due to the differences in their steric fit in the chiral cavities [10,21,23].

The separations of the three pairs of compounds on the different columns using hexane and an alcohol modifier (IPA or ethanol) as the mobile phase are shown in Figs. 2–7.

3.1. Separation of Compounds A and A′

3.1.1. Separations with hexane and IPA as the mobile phase

Compounds A and A′ yielded different degrees of separation on the two amylose-based CSPs (Chiralpak AS and Chiralpak AD) using hexane and IPA

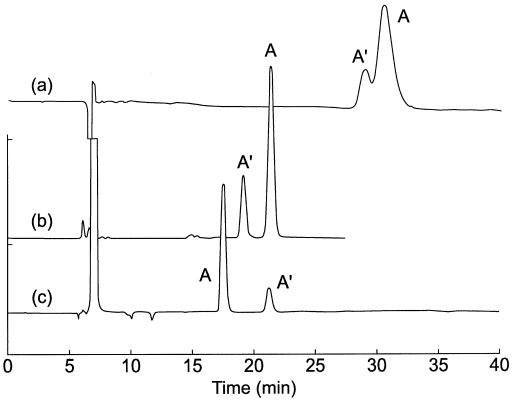

Fig. 2. Chromatograms of the separation of Compounds A and A′ using hexane–IPA as the mobile phase. HPLC conditions: (a) Chiralpak AS column (250×4.6 mm) with hexane–IPA (75:25, v/v) as the mobile phase; (b) Chiralpak AD column (250×4.6 mm) with hexane–IPA (90:10, v/v) as the mobile phase; (c) Chiralcel OD column (250×4.6 mm) with hexane–IPA (80:20, v/v) as the mobile phase. Flow-rate: 0.5 ml/min at room temperature (~22°C); UV detection: 260 nm for (a) and (c), 220 nm for (b).

as mobile phase with the minor enantiomer A′ being eluted first on both columns (Fig. 2a and b). The amylose tris(3,5-dimethylphenylcarbamate) (Chiralpak AD) column provided better separation in less time compared to the amylose tris[(*S*)-1-phenylethylcarbamate] (Chiralpak AS) column. It has been reported that there are differences in chiral recognition ability between the phenylcarbamate derivatives and the tris[(*S*)-1-phenylethylcarbamate] derivative of amylose [20,21,23,24]. The derivatiza-

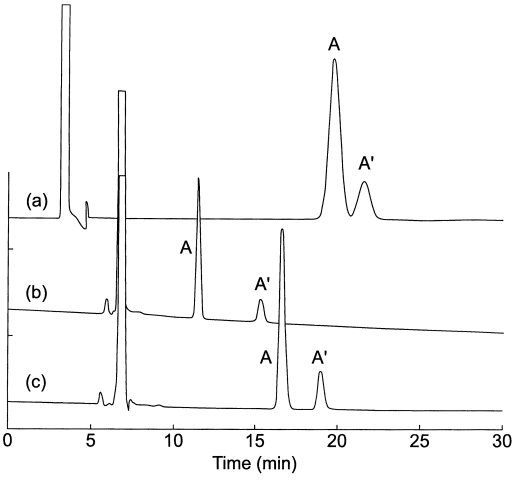

Fig. 3. Chromatograms of the separation of Compounds A and A′ using hexane–ethanol as the mobile phase. HPLC conditions: (a) Chiralpak AS column (250×4.6 mm) with hexane–ethanol (94:6, v/v) as the mobile phase; (b) Chiralpak AD column (250×4.6 mm) with hexane–ethanol (60:40, v/v) as the mobile phase; (c) Chiralcel OD column (250×4.6 mm) with hexane–ethanol (85:15, v/v) as the mobile phase. Flow-rate: 1.0 ml/min for (a), 0.5 ml/min for (b) and (c) at room temperature (~22°C); UV detection: 220 nm for (a), 260 nm for (b) and (c).

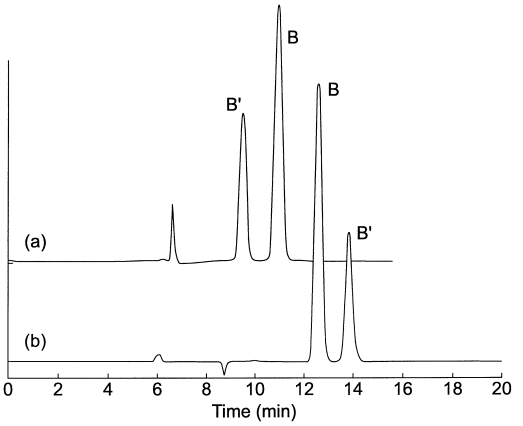

Fig. 4. Chromatograms of the separation of Compounds B and B′ using hexane–IPA as the mobile phase. HPLC conditions: (a) Chiralpak AD column (250×4.6 mm) with hexane–IPA (98:2, v/v) as the mobile phase; (b) Chiralcel OD column (250×4.6 mm) with hexane–IPA (90:10, v/v) as the mobile phase. Flow-rate: 0.5 ml/min at room temperature (~22°C); UV detection: 220 nm.

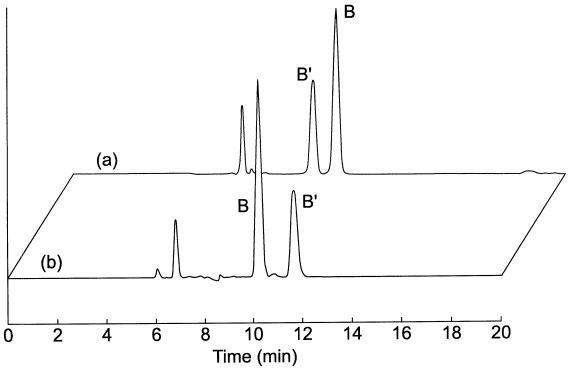

Fig. 5. Chromatograms of the separation of Compounds B and B′ using hexane–ethanol as the mobile phase. HPLC conditions: (a) Chiralpak AD column (250×4.6 mm) with hexane–ethanol (98:2, v/v) as the mobile phase; (b) Chiralcel OD column (250×4.6 mm) with hexane–ethanol (92:8, v/v) as the mobile phase. Flow-rate: 0.5 ml/min at room temperature (~ 22°C); UV detection: 260 nm.

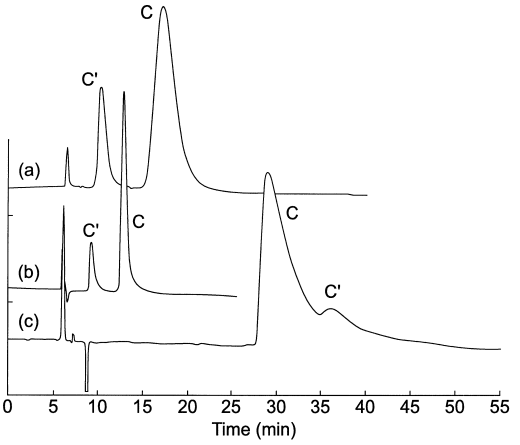

Fig. 6. Chromatograms of the separation of Compounds C and C′ using hexane–IPA as the mobile phase. HPLC conditions: (a) Chiralpak AS column (250×4.6 mm) with hexane–IPA (75:25, v/v) as the mobile phase; (b) Chiralpak AD column (250×4.6 mm) with hexane–IPA (85:15, v/v) as the mobile phase; (c) Chiralcel OD column (250×4.6 mm) with hexane–IPA (90:10, v/v) as the mobile phase. Flow-rate: 0.5 ml/min at room temperature (~22°C); UV detection: 220 nm.

tion groups on the Chiralpak AD and Chiralpak AS had significant structural differences. Compared to Chiralpak AS, the Chiralpak AD had two methyl groups on the phenyl ring, to increase the bulkiness of the aromatic functionality. In addition, the phenyl ring on Chiralpak AD was closer to the amylose ring by one carbon, therefore enhancing the bulkiness of the environment around the amylose ring. These differences could lead to the difference in higher order structure between the two CSPs, resulting in the difference in chiral recognition ability.

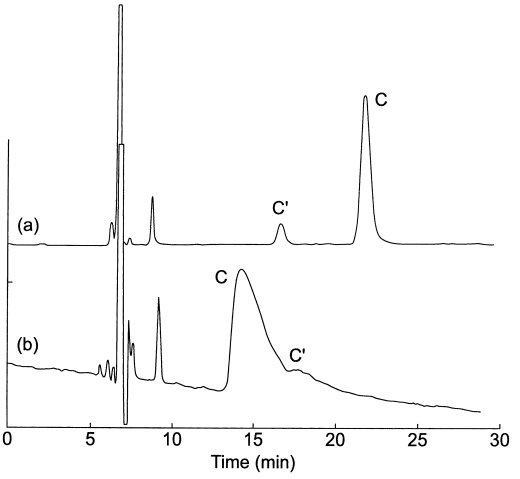

Fig. 7. Chromatograms of the separation of Compounds C and C′ using hexane–ethanol as the mobile phase. HPLC conditions: (a) Chiralpak AD column (250×4.6 mm) with hexane–ethanol (90:10, v/v) as the mobile phase; (b) Chiralcel OD column (250×4.6 mm) with hexane–ethanol (85:15, v/v) as the mobile phase. Flow-rate: 0.5 ml/min at room temperature (~22°C); UV detection: 220 nm for (a) and 260 nm for (b).

The separation of A and A′ was also performed on a Chiralcel OD column, the cellulose-based counterpart of the Chiralpak AD column. On the OD column, the derivatization groups on the cellulose were the same [tris(3,5-dimethylphenylcarbamate)] as those on the amylose-based AD column. However, the elution order of the two compounds on the OD column was reversed compared to that on the AD column (Fig. 2c). In addition, within similar analysis times, the OD column provided improved separation ($\alpha = 1.32$, $R_s = 5.2$) compared to that on the AD column ($\alpha = 1.17$, $R_s = 3.0$).

Okamoto's research group has reported numerous examples in which Chiralcel OD and Chiralpak AD columns showed different chiral recognition abilities, as well as different elution orders of many enantiomeric pairs on the two columns [21,23,24]. Okamoto et al. [21,23] attributed the difference in chiral recognition ability between the two CSPs to the conformational difference between them. Our observation on the difference in chiral recognition ability between the Chiralcel OD and Chiralpak AD columns is believed to be due to the same reason.

3.1.2. Separations with hexane and ethanol as the mobile phase

When the mobile phase was changed from hexane–IPA to hexane–ethanol, the separation of compounds A and A′ on Chiralpak AS and AD columns changed dramatically. While the enantiomers were separated on both columns (Fig. 3a and b), the elution order of the enantiomers was reversed on both columns compared to the elution with hexane–IPA as the mobile phase. The change was especially dramatic on the AD column, as shown by the change in the separation factor from 1.17 (Fig. 2b) to −1.70 (Fig. 3b), where the minus sign represents the reversed elution order. The reversal of the elution order of enantiomers on cellulose-based CSPs upon changing the alcohol modifiers in the mobile phase

has been reported by a number of research groups [12,13,28]. The authors attributed the reversal of elution order to an alteration in the steric environment of the chiral cavities caused by the change of alcohol modifiers. The reversal of the elution order of Compounds A and A′ upon changing the alcohol modifier from IPA to ethanol, in our case, was probably due to the same reason.

Another interesting phenomenon was observed on the Chiralpak AD column when the mobile-phase modifier was changed from IPA to ethanol. Since the polarity of ethanol (P' value, 4.3) is larger than that of IPA (P' value, 3.9) [29], it is expected that the k' value obtained using ethanol as the modifier would be smaller than that obtained using IPA as the modifier at the same molar concentration, if solvent polarity is the only factor in determining the k' value. However, our experimental results (Table 1) indicated that, on the AD column even with a higher molar concentration of ethanol modifier, the k' values of Compounds A and A′ were larger than those obtained using IPA as the modifier, suggesting that the polarity of the mobile phase modifier was not the dominating factor in determining the k' of the solutes on the AD column. The increased retention obtained with ethanol modifier was probably also associated with an alteration of the steric nature of the CSP.

On the Chiralcel OD column on the other hand, the elution order obtained with a hexane–ethanol mobile phase remained unchanged (Fig. 3c) compared to that obtained using a hexane–IPA mobile phase (Fig. 2c). In addition, the k' values of the solutes obtained with ethanol modifier were smaller than those obtained with IPA modifier at the same molar concentration (Table 1), which was consistent with the assumption that the polarity of the mobile-phase modifier controlled the k' of the solutes.

3.2. Separation of Compounds B and B′

Compound B has two chiral centers, as shown in Fig. 1. The 4-fluorophenyl group and the 3,5-bis-(trifluoromethyl)benzoate group are in a *cis* configuration on the morpholine ring. The simultaneous reversal of both chiral centers gave the enantiomer Compound B′. Two other possible stereoisomers having the two aforementioned functional groups in the *trans* configuration on the morpholine ring are enantiomers to each other, but are diastereomers to Compounds B and B′. In a reversed-phase achiral HPLC method, the two *trans* stereoisomers could be eluted as one peak that was separated from the peak of Compounds B and B′, and our results obtained using this method indicated that the combined level of the two *trans* stereoisomers was typically $<0.05\%$ in the purified samples of Compound B [30]. Therefore, our chiral separation was focused on the separation of the two *cis* enantiomers.

Separation of Compounds B and B′ was performed on the AD and OD columns with hexane–IPA and hexane–ethanol as the mobile phase, respectively. In all cases, baseline separation was achieved (Figs. 4 and 5); similar resolution and column performance were obtained. The elution order of the enantiomers on the AD column was reversed on the OD column. This again was believed to be due to the conformational differences between the two CSPs.

3.3. Separation of Compounds C and C′

Compound C has three chiral centers, as shown in Fig. 1. Although, theoretically, seven other stereoisomers of Compound C might exist, only one stereoisomer was possibly present in samples of Compound C based on the synthetic route used to prepare Compound C. Compound C was synthesized

Table 1
Effect of alcohol modifiers on the retention factors of compounds A and A′ on Chiralpak AD and Chiralcel OD columns

Mobile-phase modifier	AD column	OD column
IPA	Modifier concentration: 1.3 *M* k': 2.24, 2.61; R_s: 3.0	Modifier concentration: 2.6 *M* k': 2.11, 2.79; R_s: 5.2
Ethanol	Modifier concentration: 1.7 M k': 3.30, 5.55; R_s: 8.6	Modifier concentration: 2.6 *M* k': 1.96, 2.38; R_s: 3.7

from Compound B, partly by converting the C=O group on Compound B into a -CH(CH_3)- group on Compound C to form the third chiral center. The configurations of the two chiral centers on the morpholine ring of Compound C were controlled at the stage of Compound B (typical chiral purity of Compound B>99.9%). Trace amounts of Compound B′ (<0.1%) and *trans* stereoisomers of Compound B (total <0.05%) might generate other stereoisomers of Compound C during the formation of the third chiral center. However, these stereoisomers were very unlikely to be present in the sample of Compound C as they were rejected into the mother liquors during the subsequent synthesis and purification steps. Therefore, the only possible stereoisomer was generated from Compound B during the formation of the third chiral center. The configuration of the third chiral center on this stereoisomer was reversed from that on Compound C, while the configurations of the two chiral centers on the morpholine ring remained the same as those on Compound C. This α-methyl diastereomer of Compound C could be monitored by a reversed-phase achiral HPLC method and it was typically present in purified samples of Compound C at an approximate level of 0.1% [30]. Although Compound C′ (whose three chiral centers were simultaneously reversed compared to those on Compound C) was very unlikely to be present in samples of Compound C, as discussed above, it was still desirable to have a method to collect analytical data on this enantiomer of Compound C during drug development in order to address any regulatory concerns. Therefore, the development of a chiral separation method for Compounds C and C′ was carried out.

Separation of Compounds C and C′ was first performed on the AS, AD and OD columns with hexane–IPA as the mobile phase. On the amylose-based columns (AS and AD), baseline separation was achieved in each case (Figs. 6a and b). On the cellulose-based Chiralcel OD column, the resolution was poor due to low column efficiency (Fig. 6c). The elution order of the enantiomers on the Chiralcel OD column was again reversed compared to that on the two amylose-based columns.

The separations were then performed on the AD and OD columns with hexane–ethanol as the mobile phase (Fig. 7a and b). The set of conditions shown in Fig. 7a using the AD column provided the best separation ($\alpha=1.48$ and $R_s=5.4$) and was apparently the best choice among the various sets of conditions used. Under these conditions, the aforementioned α-methyl diastereomer of Compound C eluted between Compounds C′ and C, with baseline separation (chromatogram not shown), and did not interfere with the quantitation of either Compound C′ or C. On the OD column, the resolution was very poor, due to low column efficiency. The elution order of the enantiomers on the OD column was reversed compared to that on the AD column.

More experimental results indicated that, on the AD column, with 2.6 *M* ethanol modifier in the mobile phase, the k' values of Compounds C′ and C were larger than those obtained using IPA as the modifier at a lower molar concentration (Table 2). As discussed earlier for Compounds A and A′, this kind of phenomenon again suggested that the polarity of the mobile-phase modifier was not the dominating factor in determining the k' of the solutes. The increased retention obtained with ethanol modifier could again be due to an alteration of the steric nature of the CSP by ethanol.

3.4. Effect of structural variation of the solutes on their k′ values

Some general trends regarding the k' values of the three structurally related enantiomeric pairs were observed from the data given in Table 3. Compounds C and C′ showed smaller k' values than Compounds

Table 2
Effect of alcohol modifiers on the retention factors of compounds C and C′ on a Chiralpak AD column

Mobile-phase modifier	Modifier concentration (*M*)	k'	R_s
IPA	2.0	0.55, 1.15	4.1
Ethanol	2.6	1.22, 1.79	4.5

Table 3
Effect of structural variation of solutes on their k' values

Enantiomeric pair	AS column	AD column	OD column
Hexane–IPA mobile phase			
A, A′	MP: 75:25 k': 3.58, 3.82; R_s: 0.69	MP: 90:10 k': 2.24, 2.61; R_s: 3.0	MP: 90:10 k': 3.92, 5.05; R_s: 5.7
B, B′	–	MP: 98:2 k': 0.60, 0.85; R_s: 2.7	MP: 90:10 k': 1.08, 1.29; R_s: 2.5
C, C′	MP: 75:25 k': 0.60, 1.68; R_s: 2.5	MP: 90:10 k': 1.04, 2.17; R_s: 4.3	MP: 90:10 k': 3.85, 5.02; R_s: nc
Hexane–ethanol mobile phase			
A, A′	–	MP: 90:10 k': 3.30, 5.55; R_s: 8.6	MP: 85:15 k': 1.96, 2.38; R_s: 3.7
B, B′	–	MP: 98:2 k': 0.63, 0.78; R_s: 2.4	MP: 92:8 k': 0.81, 1.07; R_s: 3.7
C, C′	–	MP: 90:10 k': 1.76, 2.61; R_s: 5.4	MP: 85:15 k': 1.55, 2.15; R_s: nc

MP, mobile-phase composition (hexane–IPA or hexane–ethanol, v/v); nc, resolution was not calculated due to lack of accuracy caused by poor resolution.

A and A′ under the same mobile phase conditions. However, compared to the k' values of Compounds B and B′, the k' values for Compounds C and C′ were larger when the same or higher concentration of alcohol modifier was used. These trends indicated that under identical mobile-phase conditions, the degree of retention of the compounds was in the order of A>C>B.

Wainer et al. [10] demonstrated that steric factors of a solute played an important role in the fit of the solute into the chiral cavity of the CSP. Compared to Compound A, Compound B has the following differences: (1) increased bulkiness due to the presence of the bis(trifluoromethyl)phenyl group and (2) an ester group on Compound B replaces the carbonyl group on Compound A. The increased bulkiness of Compound B may have resulted in a lower degree of inclusion of the compound in the chiral cavities. In addition, unlike the carbonyl group on Compound A, the C=O group on Compound B has reduced electron negativity on the oxygen due to the conjugative electron-withdrawing effect of the bis(trifluoromethyl)phenyl group. This in turn could result in weaker hydrogen bonding between the C=O group on Compound B and the NH group on the CSP. The combination of these two factors could be the cause of the reduced retention of Compound B.

The retention of Compound C was between those of Compounds A and B. Compared to Compound A, Compound C has increased bulkiness, which could lead to reduced inclusion in the chiral cavity on the CSP; the absence of the carbonyl group on the upper portion of the molecule of Compound C eliminated hydrogen bonding and/or dipole–dipole interactions between that part of the molecule and the CSP. The combination of these factors could be the reason that Compound C has weaker retention than Compound A. However, Compound C showed stronger retention than Compound B, probably due to the substitution of the benzyl group on Compound B with the heterocyclic group on Compound C. Since the heterocycle on Compound C has NH and C=O functionalities, which could interact with the carbamate groups on the CSP through hydrogen bonding and/or dipole–dipole interactions, the stronger retention of Compound C compared to that of Compound B could be due to such interactions.

3.5. Comparison of the elution order of enantiomers on AD and OD columns

It is noticeable from Figs. 2 to 7 that, in five out of the six cases, the amylose-based Chiralpak AD column and its cellulose-based counterpart, the Chiralcel OD column, provided different elution orders for each pair of enantiomers. In the separation of Compounds A and A′ using ethanol as the mobile-phase modifier, the elution order of the enantiomers

was the same on both the AD and the OD columns. However, this was a special case in which the use of ethanol modifier reversed the elution order of the enantiomers on the AD column, as discussed earlier. Therefore, in the usual cases, the AD and OD columns provided different elution orders of the enantiomers. This observation can be a useful guide for future method development in which a reversal of elution order is desired.

To test the usefulness of this guide, we performed separations of a pair of enantiomers (Compounds D and D′, Fig. 8), which were not structurally related to any of the three aforementioned enantiomeric pairs. The separation of Compounds D and D′ on the AD and OD columns is shown in Figs. 9 and 10. Again, the elution order of the enantiomers was different on the AD and OD columns, with either IPA or ethanol as the polar modifier in the mobile phase. In an earlier study, we reported the separation of Compounds D and D′ on a silica-bonded polyacrylamide CSP [31]. However, in that separation, the minor enantiomer (Compound D′) eluted after the major enantiomer (Compound D); the detection limit (0.3%) of the minor enantiomer was compromised by interference from the tail of the major enantiomer. The reversal of the elution order achieved in this current study would allow a lower detection limit (0.1%) of the minor enantiomer.

3.6. Comparison of the retention of enantiomers on AD and OD columns

Besides comparing the chiral recognition ability of the Chiralpak AD and Chiralcel OD columns, it was also of interest to compare the retention of the solutes on these CSPs, which had the same derivatization group on the amylose and cellulose, respectively. Table 4 compares the retention factors of three pairs of enantiomers on the two columns. In five of the six cases shown in Table 4, the OD column showed stronger retention of the solutes

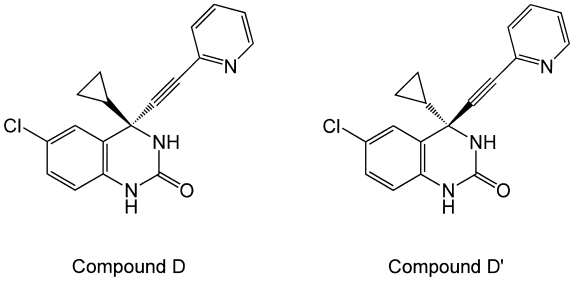

Fig. 8. Structures of Compounds D and D′.

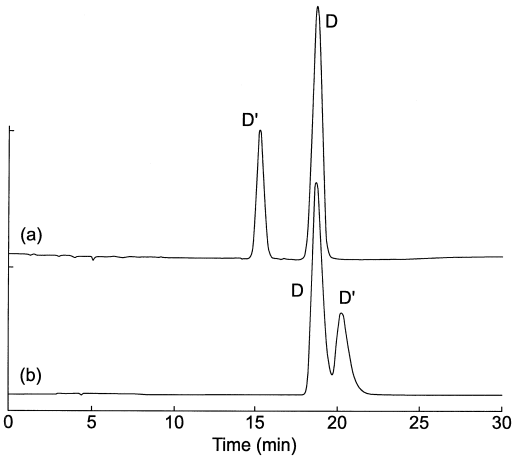

Fig. 9. Chromatograms of the separation of Compounds D and D′ using hexane–IPA as the mobile phase. HPLC conditions: (a) Chiralpak AD column (250×4.6 mm) with hexane–IPA (81:19, v/v) as the mobile phase; (b) Chiralcel OD column (250×4.6 mm) with hexane–IPA (90:10, v/v) as the mobile phase. Flow-rate: 0.5 ml/min at room temperature (~22°C); UV detection: 220 nm.

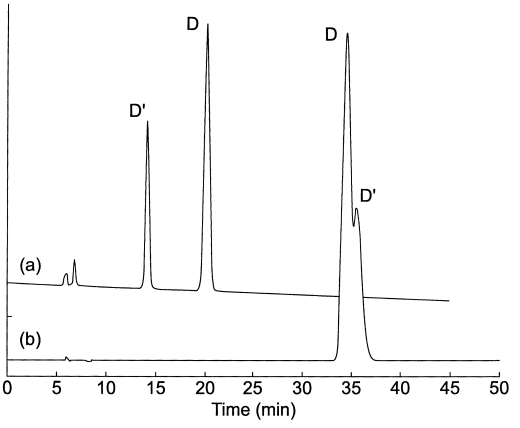

Fig. 10. Chromatograms of the separation of Compounds D and D′ using hexane–ethanol as the mobile phase. HPLC conditions: (a) Chiralpak AD column (250×4.6 mm) with hexane–ethanol (60:40, v/v) as the mobile phase; (b) Chiralcel OD column (250×4.6 mm) with hexane–ethanol (92:8, v/v) as the mobile phase. Flow-rate: 0.5 ml/min at room temperature (~22°C); UV detection: 220 nm.

Table 4
Comparison of the retention factors on Chiralpak AD and Chiralcel OD columns

Enantiomeric pair	AD column	OD column
Hexane–IPA mobile phase		
A, A′	MP: 90:10	MP: 90:10
	k': 2.24, 2.61; R_s: 3.0	k': 3.92, 5.05; R_s: 5.7
B, B′	MP: 98:2	MP: 90:10
	k': 0.60, 0.85; R_s: 2.7	k': 1.08, 1.29; R_s: 2.5
C, C′	MP: 90:10	MP: 90:10
	k': 1.04, 2.17; R_s: 4.3	k': 3.85, 5.02; R_s: nc
Hexane–ethanol mobile phase		
A, A′	MP: 85:15	MP: 85:15
	k': 2.36, 4.00; R_s: 8.3	k': 1.96, 2.38; R_s: 3.7
B, B′	MP: 98:2	MP: 92:8
	k': 0.63, 0.78; R_s: 2.4	k': 0.81, 1.07; R_s: 3.7
C, C′	MP: 85:15	MP: 85:15
	k': 1.22, 1.79; R_s: 4.5	k': 1.55, 2.15; R_s: nc

MP, mobile-phase composition (hexane–IPA or hexane–ethanol, v/v); nc, resolution was not calculated due to a lack of accuracy caused by poor resolution.

compared to its amylose-based counterpart (AD column). Since the derivatization group on both CSPs was the same, the different retention behaviors of the two CSPs should be due only to the conformational differences between the two CSPs. This suggests that the retention of solutes not only depends on the derivatization groups on the CSP, but also depends on the higher order structure of the CSP.

There is one case in Table 4 where Compounds A and A′ showed stronger retention on the AD column than on the OD column when ethanol was used as the modifier. However, this was not surprising considering the previously discussed fact that even the elution order of Compounds A and A′ on the AD column was reversed upon changing the mobile-phase modifier from IPA to ethanol. The increased retention of Compounds A and A′ on the Chiralpak AD column could be associated with the speculated alteration of the steric nature of the CSP by ethanol.

4. Conclusion

The separation of the enantiomeric pairs A/A′, B/B′, C/C′ and D/D′ was successfully achieved using Chiralcel OD, Chiralpak AD or Chiralpak AS columns. In many cases, satisfactory separation of each pair of enantiomers was achieved on more than one column. The results demonstrated the effectiveness of the tris(3,5-dimethylphenylcarbamate) derivatives of cellulose (Chiralcel OD) and amylose (Chiralpak AD) as CSPs. The amylose tris[(*S*)-1-phenylethylcarbamate] (Chiralpak AS) showed different chiral recognition abilities for some enantiomeric pairs compared to the amylose tris(3,5-dimethylphenylcarbamate) (Chiralpak AD). A reversal of the elution order was observed in the separation of Compounds A and A′ on the Chiralpak AS and AD columns when the mobile-phase modifier was changed from IPA to ethanol. The polarity of the mobile-phase modifier was not necessarily the dominating factor in determining the k' of a solute. The retention of a solute depends on its bulkiness and polar functional groups, such as C=O and NH. The amylose-based Chiralpak AD column and its cellulose-based counterpart, Chiralcel OD, retained the solutes differently although the derivatization group on both CSPs was the same. This suggests that the retention of solutes not only depends on the derivatization group on the CSP, but also depends on the higher order structure of the CSP. The Chiralpak AD and Chiralcel OD columns usually provided different elution orders of the enantiomers in our studies. This observation can be used as a crude guide for future method development in which a reversal of elution order of the enantiomers is

desirable. However, since our studies were limited, the scope of this finding will be further examined using other chiral compounds.

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