### *5.3.2 Alcoholic Mobile Phase Modifiers*

To further understand the chiral recognition mechanism governing the enantio-separation of profens on Chiralpak AD and Chiralcel OD, a study on the effect of alcoholic mobile phase modifiers was conducted. The mobile phase modifiers chosen were the alcohols that are commonly used as solvents, ethanol, isopropanol, and *tert*-butanol. The first part of the study was to investigate the influence of the concentration of the alcoholic modifiers on retention and enantisoelectivity. Racemic ketoprofen, flurbiprofen, fenoprofen, and ketoprofen methyl ester were separated on Chiralpak AD at various concentrations of the alcoholic modifier at fixed concentration of TFA. The second part was to explore the effect of the nature of the alcoholic modifier on retention and enantioselectivity of profens on Chiralpak AD and Chiralcel OD. To have a better understanding of the influence of various alcoholic modifiers on chiral recognition, aromatic hydrocarbons of benzene, naphthalene, and anthracene (Fig. 37) were included in the study. The aromatic hydrocarbons were chromatographed at varying v/v % of ethanol and hexane, but at a fixed concentration of TFA ($1.95 \times 10^{-6}$ M) at 25°C. Whereas the enantioseparations of profens were performed at constant molar concentrations of alcohol and TFA in hexane. This was to insure that the number of alcoholic modifier molecules competing with the analytes for active sites on the CSP were equal.

#### *5.3.2a Concentration of Alcoholic Mobile Phase Modifier*

As shown in Fig. 38, the consecutive increases in the concentration of ethanol in the mobile phase from 2.5 to 20% resulted in corresponding decreases in retention. The magnitude of the change of $k$ steadily diminished from an average of 45% when the ethanol content of the mobile phase was raised from 2.5 to 5% to an average of 20% decrease in $k$ for an increase in ethanol concentration from 15 to 20%. This decrease in retention is to be expected because the polarity of the mobile phase is increased when the ethanol concentration is increased.

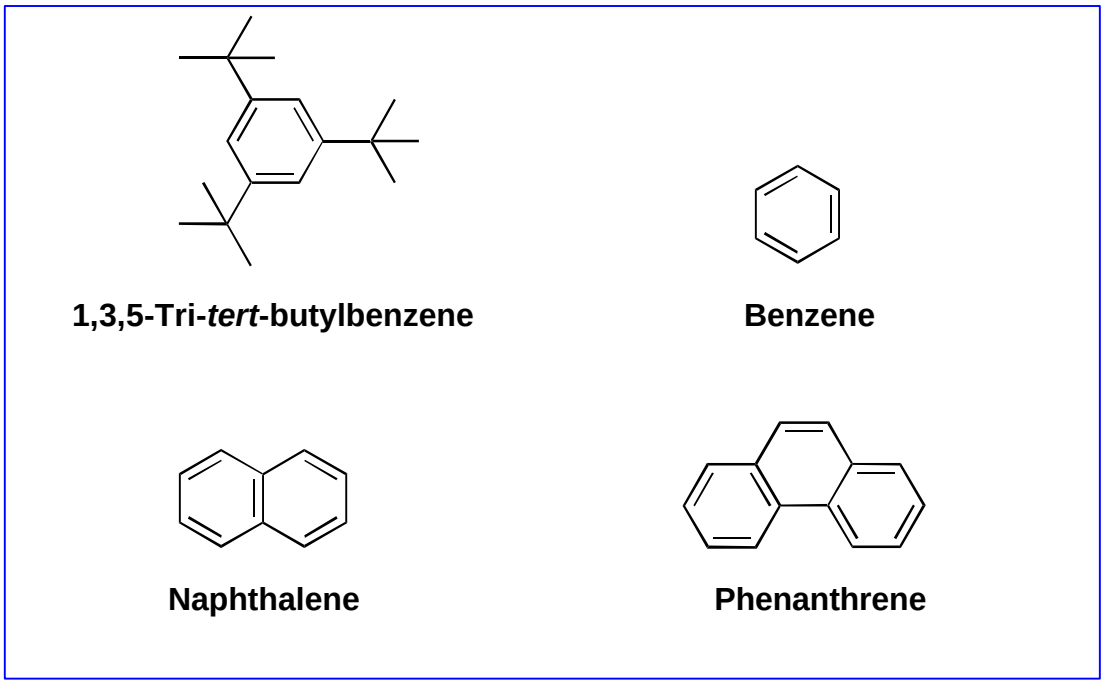


Figure 37. Aromatic hydrocarbons used as additional probe analytes with 1,3,5-tri-*tert*-butylbenzene for the determination of the column dead volume.

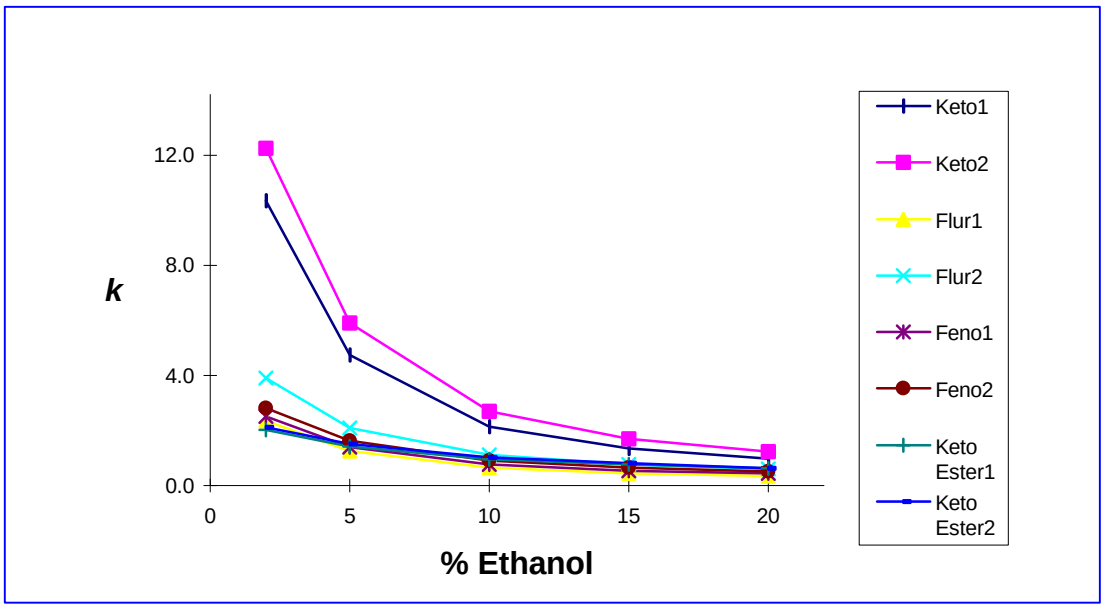


Figure 38. The effect of increasing the ethanol concentration in the mobile phase on retention on Chiralpak AD. Each data point is the mean of four replicate injections. Retention factor (***k***) error: $\pm$ 1.63 x $10^{-4}$ - $\pm$ 2.71 x $10^{-3}$.

**Table XI. The effect of the concentration of ethanol on the enantioselectivity of profens on Chiralpak AD.**

| % Ethanol | Enantioselectivity, $\alpha$ | | | |
|---|---|---|---|---|
| | Fenoprofen | Flurbiprofen | Ketoprofen | Ketoprofen Methyl Ester |
| 2.5 | 1.12 $\pm 4.75 \times 10^{-4}$ | 1.67 $\pm 6.11 \times 10^{-4}$ | 1.19 $\pm 1.14 \times 10^{-4}$ | 1.04 $\pm 4.75 \times 10^{-4}$ |
| 5 | 1.17 $\pm 8.29 \times 10^{-4}$ | 1.68 $\pm 1.69 \times 10^{-3}$ | 1.24 $\pm 2.36 \times 10^{-3}$ | 1.06 $\pm 4.75 \times 10^{-4}$ |
| 10 | 1.21 $\pm 1.05 \times 10^{-3}$ | 1.70 $\pm 1.20 \times 10^{-4}$ | 1.27 $\pm 6.02 \times 10^{-4}$ | 1.06 $\pm 4.75 \times 10^{-4}$ |
| 15 | 1.20 $\pm 5.59 \times 10^{-4}$ | 1.69 $\pm 7.60 \times 10^{-4}$ | 1.26 $\pm 6.49 \times 10^{-4}$ | 1.06 $\pm 4.75 \times 10^{-4}$ |
| 20 | 1.17 $\pm 4.51 \times 10^{-4}$ | 1.76 $\pm 5.31 \times 10^{-3}$ | 1.27 $\pm 6.65 \times 10^{-4}$ | 1.06 $\pm 1.55 \times 10^{-4}$ |

Average of six runs, 25°C, flow rate 1.0 mL/min.

The enantioselectivity of analytes was not markedly affected by the increase in the alcoholic modifier's concentration as shown in Table XI, except for ketoprofen and fenoprofen which is about 7%. The enantioselectivity of ketoprofen methyl ester was unchanged, except when ethanol was 2.5% in the mobile phase.

The above results strongly indicate that the alcoholic modifier molecules, ethanol in this case, compete with the analytes for achiral and chiral adsorption sites of the CSP. Thus, the retention and enantioselectivity were altered by changes of the concentration of ethanol in the mobile phase.

### *5.3.2b Influence of Alcoholic Mobile Phase Modifiers on Aromatic Hydrocarbons*

The results for the retention of aromatic hydrocarbons are presented on Table XII. Considering the retention of 1,3,5-tri-*tert*-butylbenzene, which is used to determine the column's dead volume, all aromatic hydrocarbons were slightly retained on Chiralpak AD. The retention seems to dependent on their size. The increasing order of their retention is benzene > naphthalene > anthracene. This trend corresponds to the increasing order of their molecular size. In addition, although there was a decreasing trend in the retention when the alcoholic modifier was changed from ethanol to 2-propanol to *tert*-butanol, the change in retention is noticeable for phenanthrene. This decreasing trend of retention is not due to the polarity of the mobile phase because with ethanol, which is the most polar among the alcoholic modifiers, the analytes have the longest retention. This trend, however, can be correlated to the increasing order of the steric size of the alcoholic modifier: ethanol, 2-propanol, and *tert*-butanol.

These results strongly suggest that the analytes were retained on Chiralpak AD by insertion into the chiral cavity. Their retention seems to be dependent on their fit to the chiral cavities. The aromatic hydrocarbons are very nonpolar and the only possible interactions with the polar CSP is by London dispersion forces.

The retention of the aromatic hydrocarbons on Chiralcel OD are presented in Table XIII. The aromatic hydrocarbons, with reference to 1,3,5-tri-*tert*-butylbenzene were also slightly retained on Chiralcel OD. The trend of retention is the same order for increasing molecular size: benzene, naphthalene, and phenanthrene. These results also strongly indicate that the mechanism for their retention is by insertion of the aromatic portion to the chiral cavity of the CSP.

### *5.3.2c Nature of the Alcoholic Mobile Phase Modifiers*

The properties of the various alcoholic modifiers are shown in Table XIV. The polarity index, P', according to Rohrschneider[287] is an indication of the ability of the

**Table XII. The effect of various alcoholic modifiers on the retention of aromatic hydrocarbons on Chiralpak AD.**

| Analyte | Retention Time (min) | | |
|---|---|---|---|
| | Alcoholic Modifier (3.42 M EtOH in hexane with 1.95 x $10^{-6}$ moles TFA) | | |
| | Ethanol | 2-Propanol | *Tert*-butanol |
| 1,3,5-Tri-*tert*-butylbenzene | 3.04 ± 5.33 x $10^{-8}$ | 3.01 ± 5.77 x $10^{-4}$ | 2.99 ± 5.00 x $10^{-4}$ |
| Benzene | 3.35 ± 1.17 x $10^{-3}$ | 3.31 ± 5.77 x $10^{-4}$ | 3.29 ± 8.16 x $10^{-4}$ |
| Naphthalene | 3.53 ± 1.17 x $10^{-4}$ | 3.47 ± 1.00 x $10^{-4}$ | 3.43 ± 9.57 x $10^{-4}$ |
| Phenanthrene | 4.03 ± 5.16 x $10^{-4}$ | 3.81 ± 5.77 x $10^{-4}$ | 3.76 ± 5.00 x $10^{-4}$ |

Average of four runs, 25°C, flow rate 1.0 mL/min.

**Table XIII. Retention of aromatic hydrocarbons on Chiralcel OD.**

| Analyte | Retention Time (min) |
|---|---|
| | Alcoholic Modifier (3.43 EtOH in hexane with 1.95 x $10^{-6}$ moles TFA) |
| 1,3,5-Tri-*tert*-butylbenzene | 2.77 ± 8.20 x $10^{-4}$ |
| Benzene | 3.21 ± 1.00 x $10^{-4}$ |
| Naphthalene | 3.56 ± 5.00 x $10^{-4}$ |
| Phenanthrene | 3.72 ± 5.00 x $10^{-4}$ |

Average of four runs, 25°C, flow rate of 1.0 mL/min.

**Table XIV. Polarity index, P', and selectivity parameters, X, as defined and calculated by Snyder[289] from solubility data reported by Rohrschneider.**

| Polar Solvent | Polarity Index P' | $H^+$ Acceptor $x_e$ | $H^+$ Donor $x_d$ | Strong Dipole $x_n$ |
|---|---|---|---|---|
| Ethanol | 4.3 | 0.52 | 0.19 | 0.29 |
| 2-Propanol | 3.9 | 0.55 | 0.19 | 0.27 |
| *tert*-Butanol | 4.1 | 0.56 | 0.20 | 0.24 |

solvent to take part in strong intermolecular interactions with others, like molecules. The selectivity parameters, $x_e$, $x_d$, and $x_n$, can be considered as the reflecting the ability of the solvent to function as a proton acceptor, proton donor, or strong dipole respectively. Alcohols have high proton accepting abilities, thus, are considered as essentially proton acceptors.

From Table XIV, the increasing order of the alcoholic modifiers as proton acceptors is ethanol, 2-propanol, and *tert*-butanol. This is also the order of increasing size of alcoholic modifiers.

The results of these various alcohols on retention, enantisoelectivity, and resolution of profens on Chiralpak AD and Chiralcel OD are summarized in Tables XV and XVI. As shown in Table XIII, the change in mobile phase modifier from ethanol to 2-propanol to *tert*-butanol at constant molar concentration results in a steady decrease in $k_2$ for the enantioseparation of profens on Chiralpak AD. The decreases in $k_2$ for carprofen, fenoprofen, flurbiprofen, and ketoprofen average around 63, 198, 37, and 104%, respectively.

These results are not consistent with the increasing ability of the alcohols to displace the analytes from the CSP due to an increase in alcohol polarity. The polarity index of ethanol, 2-propanol, and *tert*-butanol are 4.3, 3.9, and 4.1, respectively. Ethanol, which is

**Table XV. Summary of the enantioseparation of profens on Chiralpak AD using different alcoholic modifiers.**

| Profen | Alcoholic Mobile Phase Modifier | $k_2$* | Enantio-selectivity α | Resolution **R** |
|---|---|---|---|---|
| Carprofen (3.42 M EtOH in hexane with 1.95 x $10^{-6}$ moles TFA) | Ethanol | 2.08 ± 8.70 x $10^{-4}$ | 1.06 ± 6.18 x $10^{-4}$ | 0.67 ± 7.30 x $10^{-3}$ |
| | 2-Propanol | 1.57 ± 7.49 x $10^{-4}$ | 1.25 ± 8.90 x $10^{-4}$ | 2.11 ± 1.65 x $10^{-2}$ |
| | *tert*-Butanol | 1.03 ± 3.40 x $10^{-3}$ | 1.03 ± 4.57 x $10^{-3}$ | 0.67 ± 2.00 x $10^{-2}$ |
| Fenoprofen (0.86 M EtOH in hexane with 1.95 x $10^{-6}$ moles TFA) | Ethanol | 1.64 ± 7.04 x $10^{-4}$ | 1.17 ± 8.29 x $10^{-4}$ | 1.91 ± 8.99 x $10^{-3}$ |
| | 2-Propanol | 1.60 ± 4.20 x $10^{-4}$ | 1.30 ± 3.42 x $10^{-4}$ | 2.99 ± 6.49 x $10^{-3}$ |
| | *tert*-Butanol | 0.83 ± 6.83 x $10^{-4}$ | 1.07 ± 1.62 x $10^{-3}$ | 0.51 ± 1.23 x $10^{-2}$ |
| Flurbiprofen (0.86 M EtOH in hexane with 1.95 x $10^{-6}$ moles TFA) | Ethanol | 2.09 ± 1.72 x $10^{-3}$ | 1.68 ± 1.69 x $10^{-3}$ | 6.32 ± 2.18 x $10^{-2}$ |
| | 2-Propanol | 1.48 ± 3.26 x $10^{-4}$ | 1.55 ± 4.33 x $10^{-4}$ | 4.60 ± 7.98 x $10^{-3}$ |
| | *tert*-Butanol | 1.15 ± 7.28 x $10^{-4}$ | 1.38 ± 1.66 x $10^{-3}$ | 2.68 ± 2.57 x $10^{-2}$ |
| Ketoprofen (0.86 M EtOH in hexane with 1.95 x $10^{-6}$ moles TFA) | Ethanol | 5.90 ± 2.36 x $10^{-3}$ | 1.24 ± 5.78 x $10^{-4}$ | 3.32 ± 1.23 x $10^{-2}$ |
| | 2-Propanol | 4.11 ± 6.75 x $10^{-4}$ | 1.23 ± 3.02 x $10^{-4}$ | 2.82 ± 4.89 x $10^{-3}$ |
| | *tert*-Butanol | 2.98 ± 3.19 x $10^{-3}$ | 1.00 ± 1.52 x $10^{-3}$ | 0.00 |

*Retention factor of second eluted enantiomer

Average of six runs, 25°C, flow rate of 1.0 mL/min.

the most polar alcohol, retained the analytes the longest. Instead, the decreasing trend for retention factor is directly related with the increasing ability of the alcoholic modifier to form hydrogen bonding, as well as the increasing trend of size. The proton accepting ability for ethanol, 2-propanol, and *tert*-butanol are 0.52, 0.55, and 0.56, respectively.

When ethanol was replaced by *tert*-butanol, there was a dramatic decrease in the retention of analytes, with an average decrease of 160% in the observed retention factors (Table XVI). The percent changes in retention when ethanol was changed to *tert*-butanol for carprofen, fenoprofen, flurbiprofen, and ketoprofen were 103, 303, 59, and 176%, respectively.

There is no correlation between the enantioselectivity, $\alpha$, and the proton accepting ability, $x_e$, of alcoholic modifier for Chiralpak AD. The influence on $\alpha$ varies according to the structure of the solute. However, the enantioselectivity of profens is smaller with *tert*-butanol than with ethanol and 2-propanol (Table XV). The effect of the change of the mobile phase modifier on the enantioselectivity of profens on Chiralpak AD is less dramatic. The overall change in enantioselectivity is about 16%.

The resolution of racemic profens also exhibits the same behavior as that of enantioselectivity. Enantiomer resolution varied according to the structure of the profens. Nevertheless, the resolution was very low when *tert*-butanol was the alcoholic modifier. Ketoprofen, which had a resolution of 3.32 and 2.82 with ethanol and 2-propanol, respectively, became zero with *tert*-butanol. This was the consequence when $\alpha$ changed to 1.00 (Table V).

One plausible conclusion that can be drawn from the above results is that hydrogen bonding is the dominant interaction of profens with Chiralpak AD for retention. Hence the profens have the shortest retention with *tert*-butanol because among the alcoholic modifiers it has the highest tendency to compete with the analytes for hydrogen bonding with the CSP. Secondly, the additional or secondary hydrogen bonding appears to be critical for chiral discrimination of profens on Chiralpak AD. With *tert*-butanol, the profens have the lowest enantioselectivity values. In fact the enantioselectivity of ketoprofen changed from 1.24 and 1.23 to 1.00 (Table XV) when ethanol and 2-propanol,

**Table XVI. Percent change in retention and enantioselectivity of profens on Chiralpak AD when ethanol is replaced by *tert*-butanol as the alcoholic modifier.**

| Profen | % $k_2$ | % $\alpha$ |
|---|---|---|
| Carprofen | 102.5 | 3.2 |
| Fenoprofen | 303.3 | 20.5 |
| Flurbiprofen | 57.8 | 6.6 |
| Ketoprofen | 175.9 | 23.6 |

respectively, were changed to *tert*-butanol. Consequently, enantiomer resolution of ketoprofen was zero. These conclusions drawn for retention and enantioselectivity of profens on Chiralpak AD are similar to those obtained from the study of the effect of acidic mobile phase modifiers.

The effect of various alcoholic mobile phase modifiers on the enantioseparation ketoprofen on Chiralpak AD is shown in Fig. 39. Enantiomeric resolution of ketoprofen was not observed when *tert*-butanol, a strong hydrogen acceptor, replaced ethanol as the alcohol modifier.

As shown in Table XVII, the decreasing trend of the retention factors due to the increasing ability of the alcoholic modifier to form a hydrogen bond is not completely observed in Chiralcel OD. With the same analytes, only flurbiprofen and ketoprofen followed the trend. The decreasing trend of retention could neither be also attributed to the increasing polarity nor the size of the alcoholic modifiers. The influence of the nature of the alcoholic modifiers varied according to the structure of the analyte. Furthermore, the effect of the change of the alcoholic modifier from ethanol to *tert*-butanol on the capacity factor was less dramatic than on Chiralpak AD (Table XVIII). The changes in $k_2$ for all profens average around 12%.

The influence of the alcoholic modifier on enantioselectivity of profens on Chiralcel OD appears to be dependent on the alcohol's proton accepting ability. As shown in Table

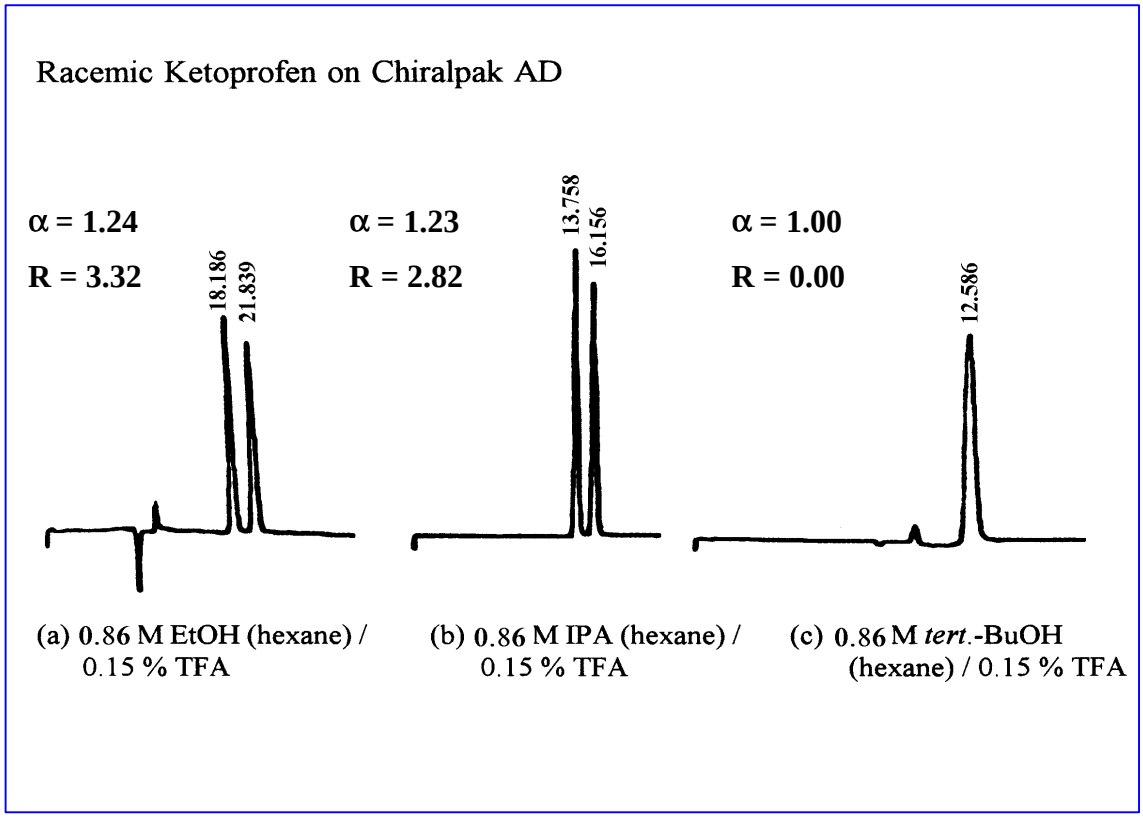


Figure 39. Influence of various alcoholic mobile phase modifiers on the chiral separation of racemic ketoprofen on Chiralpak AD. Mobile phase: 0.86 M alcohol in hexane with 1.95 x $10^{-6}$ moles TFA; flow rate: 1.0 mL/min; UV detection: 260 nm; temperature: 25°C. Individual enantioselectivity ($\alpha$) and resolution (**R**) values represent six replicate injections.

**Table XVII. Summary of the chiral separation of racemic profens on Chiralcel OD using different alcoholic modifiers.**

| Profen | Alcoholic Mobile Phase Modifier | $k_2$* | Enantio-selectivity $\alpha$ | Resolution **R** |
|---|---|---|---|---|
| Carprofen | Ethanol | $1.99 \pm 2.87 \times 10^{-3}$ | $1.15 \pm 2.62 \times 10^{-3}$ | $0.90 \pm 1.58 \times 10^{-2}$ |
| (1.72 M alcohol | 2-Propanol | $1.70 \pm 2.57 \times 10^{-3}$ | $1.22 \pm 2.23 \times 10^{-3}$ | $1.08 \pm 1.16 \times 10^{-2}$ |
| in hexane-TFA) | *tert*-Butanol | $1.80 \pm 4.20 \times 10^{-3}$ | $1.26 \pm 4.62 \times 10^{-3}$ | $1.02 \pm 1.69 \times 10^{-2}$ |
| Fenoprofen | Ethanol | $3.09 \pm 4.06 \times 10^{-3}$ | $1.12 \pm 2.18 \times 10^{-3}$ | $1.17 \pm 2.65 \times 10^{-2}$ |
| (0.34 M alcohol | 2-Propanol | $2.93 \pm 3.42 \times 10^{-3}$ | $1.15 \pm 1.97 \times 10^{-3}$ | $1.31 \pm 1.87 \times 10^{-2}$ |
| in hexane-TFA) | *tert*-Butanol | $3.33 \pm 1.52 \times 10^{-2}$ | $1.17 \pm 7.85 \times 10^{-3}$ | $1.44 \pm 1.20 \times 10^{-2}$ |
| Flurbiprofen | Ethanol | $2.15 \pm 2.59 \times 10^{-3}$ | $1.08 \pm 1.99 \times 10^{-3}$ | $0.76 \pm 1.80 \times 10^{-2}$ |
| (0.34 M alcohol | 2-Propanol | $1.95 \pm 1.75 \times 10^{-3}$ | $1.17 \pm 1.46 \times 10^{-3}$ | $1.27 \pm 1.06 \times 10^{-2}$ |
| in hexane-TFA) | *tert*-Butanol | $1.75 \pm 3.93 \times 10^{-3}$ | $1.19 \pm 4.08 \times 10^{-3}$ | $1.42 \pm 2.88 \times 10^{-2}$ |
| Ketoprofen | Ethanol | $4.77 \pm 5.26 \times 10^{-3}$ | $1.08 \pm 1.59 \times 10^{-3}$ | $0.87 \pm 1.80 \times 10^{-2}$ |
| (0.34 M alcohol | 2-Propanol | $4.50 \pm 5.40 \times 10^{-3}$ | $1.08 \pm 1.85 \times 10^{-3}$ | $0.61 \pm 1.90 \times 10^{-2}$ |
| in hexane-TFA) | *tert*-Butanol | $4.49 \pm 1.81 \times 10^{-3}$ | $1.08 \pm 5.46 \times 10^{-3}$ | $0.58 \pm 3.78 \times 10^{-2}$ |

*Retention factor of second eluted enantiomer.

Average of six runs, 25°C, flow rate of 1.0 mL/min.

**Table XVIII Percent change in retention and enantioselectivity on Chiralcel OD when ethanol is replaced by *tert*-butanol as the alcoholic modifier.**

| Profen | % $k_2$ | % $\alpha$ |
|---|---|---|
| Carprofen | 10.9 | 9.2 |
| Fenoprofen | 7.8 | 4.6 |
| Flurbiprofen | 23.9 | 8.9 |
| Ketoprofen | 6.3 | 0.6 |

XVII, the increasing trend in the enantioselectivity of profens can be correlated to the increasing ability of the alcoholic modifier to form hydrogen bonding. Enantioselectivity of all profens is greatest with *tert*-butanol and smallest with ethanol. Nevertheless, the effect of the change of mobile phase modifier on the enantioselectivity of profens was less dramatic (Table XVIII). The change in enantioselectivity of profens on Chiralcel OD average around 6%. In fact, with ketoprofen the resolution is virtually unchanged.

There is no trend on the resolution of racemic profens on Chiralcel OD. The resolution appeared not to be influenced primarily by enantioselectivity. As mentioned previously, resolution is a complicated function of $\alpha$, ***k***, and efficiency, **N**.

Figure 40 shows the influence of various alcoholic modifiers on the separation of racemic flurbiprofen on Chiralcel OD. Enantioselectivity of flurbiprofen was improved when the modifier was changed from ethanol to IPA to *tert*-butanol.

A plausible explanation for the increasing trend of enantioselectivity when the hydrogen bonding ability of the alcoholic modifier is increased may be due to the efficient interactions arising from the modification of the higher order structure of Chiralcel OD. As noted in Chapter III, mobile phase molecules with a high hydrogen bonding ability can induce a high degree of swelling of derivatized cellulose CSPs. Consequently, adsorption sites are changed due to modification of the size and geometry of the chiral cavities, thus

affecting retention and enantioselectivity. It can be inferred from the above results that the secondary hydrogen bonding may not be critical for chiral discrimination. Inclusion to the chiral cavities or other type of interactions, such as dipole-dipole stacking or π-π interaction, may be critical for the chiral recognition of profens on Chiralcel OD.

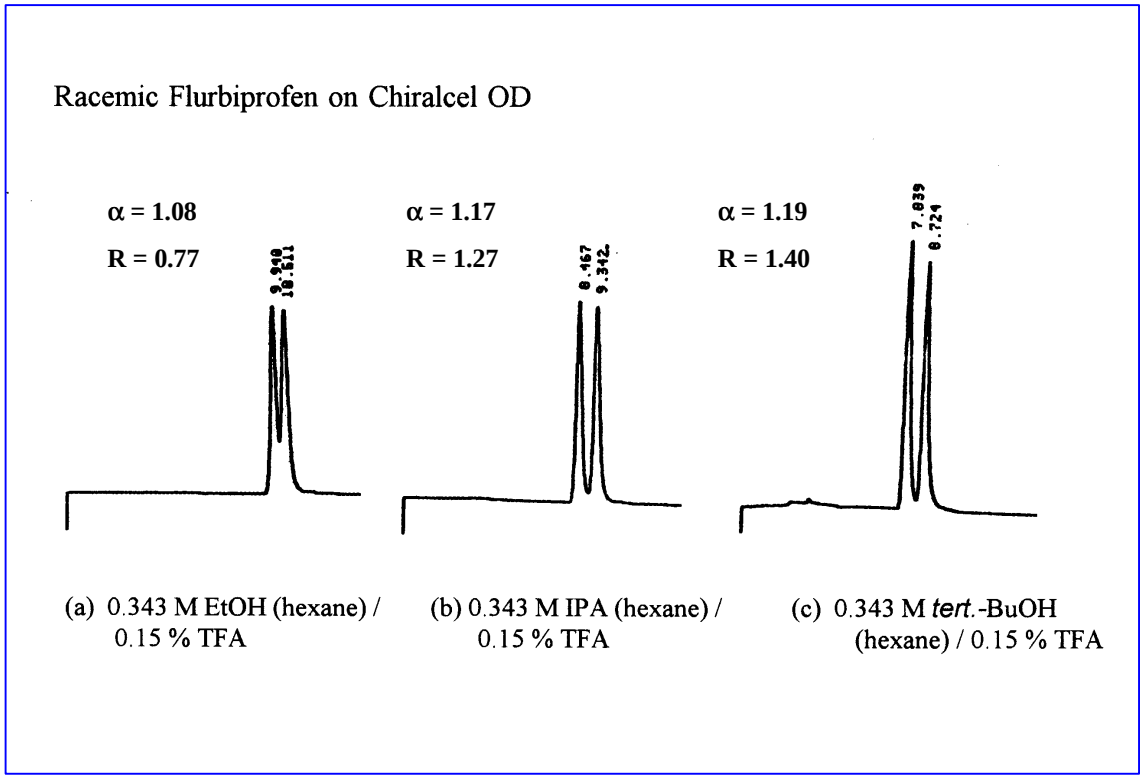


Figure 40. Influence of various alcoholic mobile phase modifiers on the separation of racemic flurbiprofen on Chiralcel OD. Mobile phase: 0.34 M alcohol in hexane with 1.95 x $10^{-6}$ moles TFA; flow rate: 1.0 mL/min; UV detection: 260 nm; temperature: 25°C. Individual enantioselectivity (**α**) and resolution (**R**) values represent six replicate injections.

## 5.4 Retention and Chiral Recognition Mechanisms

The chiral recognition process reflects all chiral and achiral interactions between racemic analyte and CSP. The results of the optimization, temperature dependence, and mobile phase studies indicated that the retention and chiral recognition mechanisms for the profens on either Chiralpak AD or Chiralcel OD are complex and interrelated

The chiral recognition mechanism for the separation of profens on Chiralpak AD, as suggested by the results, involve the following:

(1) Hydrogen bonding between the carbonyl oxygen and acid proton of the carboxyl moiety of profen and the amide proton and carbonyl oxygen of the carbamate moiety of the CSP, respectively. This is the dominant analyte-CSP interaction for retention, and for the formation of the diastereomeric analyte-CSP complexes.

(2) Insertion of enantiomers into chiral cavities, $\pi$-$\pi$ interactions between aromatic rings, and dipole-dipole interactions between the polar carbamate moiety of CSP and the polar groups of profen. These contribute to the stabilization of the diastereomeric analyte-CSP complexes.

(3) Additional or secondary hydrogen bonding that does not arise from the carboxyl moiety of the analyte. This further stabilizes the diastereomeric analyte-CSP complexes, and adds the driving force for chiral discrimination to effect enantiomeric separation.

The key role of the hydrogen bonds between the carboxyl moiety of profen and carbamate moiety of the CSP for retention and the formation of diastereomeric complexes is strongly substantiated by the drastic decrease in retention when the profens were converted to methyl esters. In addition, a decreasing trend in the retention of profens on Chiralpak AD was observed when the alcoholic mobile phase modifier was changed from ethanol to *tert*-butanol, which is the stronger proton acceptor.

The stabilization of diastereomeric complexes by insertion into chiral cavities, as well as the other interactions, has been demonstrated by the changes in retention and enantioselectivity when different acids and alcohols were used as modifiers. The acidic

and alcoholic mobile phase modifiers are recognized to alter the geometry and size of the chiral cavities of the CSP.

The driving force for chiral recognition to be the secondary hydrogen bond between profen and CSP is supported by the enantiomeric resolution of the ketoprofen ester. This result strongly suggests that the hydrogen bond coming from the keto oxygen of the profen is the primary force behind chiral recognition. Among the esters, ketoprofen methyl ester has the greatest ability to form a hydrogen bond that does not come from the carboxylate moiety. In addition, from the study of the influence of the alcoholic modifier, the enantioselectivity of profens was smallest with *tert*-butanol. In fact, ketoprofen which has the greatest ability to form additional hydrogen bond, among the profens, that does not come from the carboxyl moiety has an enantioselectivity of 1.0 when *tert*-butanol was the alcoholic modifier. The enantioselectivity of ketoprofen was 1.24 with EtOH as the alcoholic modifier. The proton accepting ability of ethanol is lower than isopropanol (0.52 and 0.56, respectively).

From the study of temperature dependence of enantioseparation of profens on Chiralpak AD, chiral separation is enthalpy controlled. This means that a strong interaction between racemic analyte-CSP, such as hydrogen bonding, mainly governs chiral discrimination. All profens have the carboxylic moiety, therefore, it is not the hydrogen bond that comes from the carboxyl moiety predominantly contributes to chiral recognition. It must be the additional hydrogen bond that does not come from the carboxyl moiety of profen. Among the profens, ketoprofen has the pronounced effect in enantioselectivity when the temperature was changed from 25 to 5 °C (Fig. 20).

This proposed mechanism for the chiral recognition process is similar to that obtained by Wainer *et al*.[288] for the separation of racemic 2-alkylarylcarboxylic acids on Chiralpak AD using QSERR. It must be noted that, as mentioned previously, most of these studies were done before Wainer *at al*. published their studies.

On Chiralcel OD, there are two chiral recognition mechanisms, entropy and enthalpy contolled. For the entropy controlled machanism, this may involve the following interactions between profen-CSP:

(1) Hydrogen bonding between the carbonyl oxygen and acid proton of the carboxyl moiety of profen and the amide proton and carbonyl oxygen of the carbamate moiety of the CSP, respectively. This is also the dominant analyte-CSP interaction for retention, and for the formation of the diastereomeric analyte-CSP complexes.

(2) Insertion of enantiomers into chiral cavities, π-π interactions between aromatic rings of profen and CSP, and dipole-dipole interactions between the polar carbamate moiety of the CSP and the polar groups of profen. These contribute to the stabilization of the diastereomeric analyte-CSP complexes.

(3) Chiral disrimination arises due to: (a) the difference in the steric fit of enantiomers in the chiral cavity of the CSP (entropy controlled); and (b) dipole-dipole or π-π interactions between enantiomer analytes and CSP (enthalpy controlled).

For the role of interactions (1) to (2) between profen and Chiralcel OD, similar explanations in Chiralpak AD could be applied.

From the results of the study on Chiralcel OD, the additional hydrogen bonding interaction is not critical for chiral recognition in either entropy or enthalpy controlled mecanism. This has been shown by the increase in enantioselectivity when the alcoholic modifier was changed from a weak (ethanol) to a strong proton acceptor (*tert*-butanol). Instead, this increasing trend of enantioselectivity due to the increasing proton accepting ability of alcohol modifiers strongly indicates that steric fit to the chiral cavities or other type of analyte-CSP interaction is critical for chiral discrimination. As noted in Chapter III, the degree of swelling of derivatized cellulose CSPs is dependent on the proton accepting ability of the alcoholic modifier. The modification of the geometry and size of the chiral cavities of Chiralcel OD by *tert*-butanol enhances chiral separation of profens.

Another proof that the additional hydrogen bonding is not critical for chiral discrimination on Chiralcel OD is the zero separation of the methyl esters of fenoprofen, ibuprofen, and ketoprofen. Considering the fact that the profen methyl esters were more retained on Chiralcel OD than on Chiralpak AD enantioseparation must be expected to take place, which was not the case.

The two different mechanisms operating for the chiral recognition of profens on Chiralcel OD are strongly substantiated by the bidirectional influence of column temperature on the enantioselectivities of profens. That is, the enantioseparations of profens with free phenyl moieties (ibuprofen, ketoprofen, and fenoprofen) were enhanced at high temperatures (entropy controlled), and the separation of racemic profens with fused rings (carprofen and naproxen) were favored at low temperatures (enthalpy controlled).

On Chiralcel OD, the entropy controlled mechanism can be explained in terms of the difference in the steric fit of enantiomer analytes in the chiral cavity of the CSP to be critical for chiral recognition. This means that the stationary phase has the preference for a certain size and shape of analyte molecule. Whereas the enthalpy controlled mechanism for the enantioseparations of carprofen and naproxen, dipole-dipole or $\pi$-$\pi$ interactions may be the driving force for chiral recognition.

The chiral recognition process for the separation of profens on Chiralcel OD is different from that on Chiralpak AD. This has been demonstrated by the different mobile phase compositions to optimize the chiral separation of profens on both CSPs. Furthermore, there was a reversal in the order of enantioselectivities (at the optimized conditions) on both CSPs. This result also indicates that the enantioseparating ability of Chiralpak AD and Chiralcel OD for the profens are complementary.