# CHAPTER V
# RESULTS AND DISCUSSION

## 5.1 Preliminary Optimization Studies

The first set of experiments was the optimization of the separation of racemic profens on Chiralpak AD and Chiralcel OD at 25°C. Enantioseparation was first tried using 80/20 and 90/10 of hexane / ethanol as a mobile phase. There was no single pair of enantiomers separated on both CSPs and the analytes were eluted at a long time (Fig.15) To facilitate the enantioseparations, as well as shorten the analysis time, TFA was added to the mobile phase. Initally, 0.1% TFA was used while the ratio of hexane and ethanol in the mobile phase was varied in an attempt to improve resolution. After achieving a maximum enantioselectivity and resolution, the ratio of hexane and ethanol was then kept constant while the composition of TFA was increased to further the separation. Relatively small amounts of TFA were added to the eluent of hexane/ethanol to optimize the chiral separations, about 0.15 - 0.40 % v/v (Table IV). Exceeding this optimum amount of TFA, meant a decrease in enantioselectivity.

For example, as shown in Fig. 15, enantioseparation of ibuprofen was optimum using 98/2/0.25% hexane/ethanol/TFA as the mobile phase. The enantioselectivity ($\alpha$) and enantiomer resolution (**R**) were 1.06 and 1.03, respectively. When TFA was further increased to 0.4%, while the %v/v ratio of hexane/ethanol remained 98/20, both $\alpha$ and **R** decreased to 1.05 and 0.64, respectively, accompanied by a decrease in the retention.

TFA may have several roles in the enantioseparation of profens on Chiralpak AD and Chiralcel OD. It interacts with the carbamate moieties of the CSP by hydrogen bonding modifying the higher order structure of the CSPs, as well as those of the profens hence the enantioseparation is enhanced. Secondly, it competes with the analytes for the active chiral and achiral adsorption sites on the CSP resulting in their quicker desorption from the CSP, thus, a shorter analysis time. And lastly, TFA may also compete with the

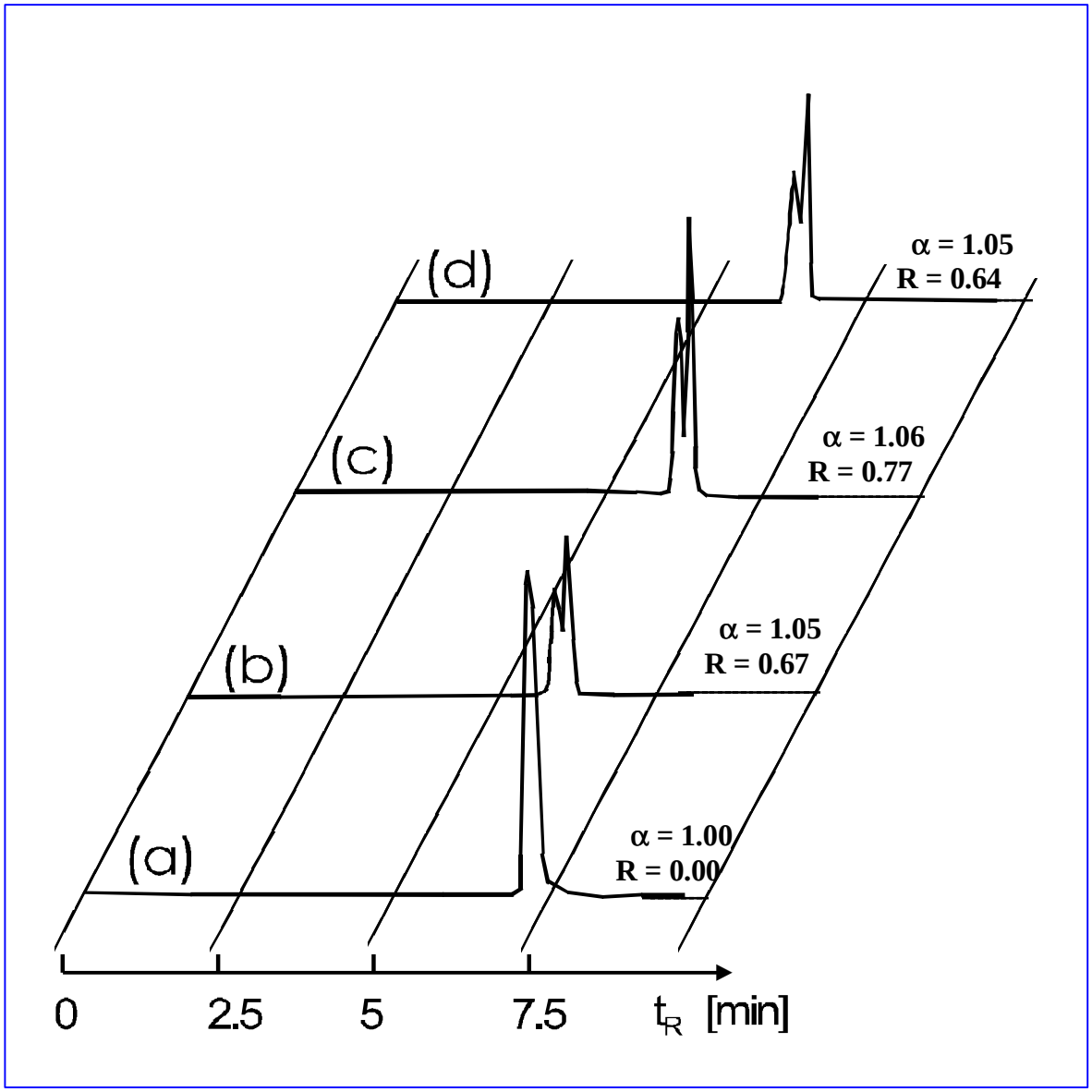


Fig. 15. Chromatograms showing the enantioseparation of racemic ibuprofen on Chiralpak AD: temperature, 25°C; flow rate, 1.0 mL/min; UV detection, 260 nm; mobile phase, hexane/ethanol/TFA: (a) 98/2/0.00, (b) 98/2/0.15, (c) 98/2/0.25, and (d) 98/2/0.40. Each enantioselectivity (α) value is the mean of three replicate injections.

**Table IV. Measured chromatographic parameters for the chiral separations of racemic profens on Chiralpak AD and Chiralcel OD.**

| Racemates | Mobile Phase Hexane*/EtOH/ TFA | $k_2$ | Enantio-selectivity $\alpha$ | Resolution **R** |
|---|---|---|---|---|
| *Chiralpak AD* | | | | |
| Carprofen | 80/20/0.15 | $2.25 \pm 2.00 \times 10^{-3}$ | $1.06 \pm 2.00 \times 10^{-3}$ | $0.65 \pm 1.00 \times 10^{-2}$ |
| Fenoprofen | 80/20/0.15 | $1.68 \pm 2.00 \times 10^{-3}$ | $1.17 \pm 5.00 \times 10^{-3}$ | $1.83 \pm 2.00 \times 10^{-2}$ |
| Ibuprofen | 98/2/0.25 | $1.31 \pm 2.00 \times 10^{-3}$ | $1.06 \pm 5.00 \times 10^{-3}$ | $1.03 \pm 7.00 \times 10^{-3}$ |
| Ketoprofen | 80/20/0.15 | $1.30 \pm 1.00 \times 10^{-3}$ | $1.28 \pm 2.00 \times 10^{-3}$ | $2.37 \pm 2.00 \times 10^{-2}$ |
| Naproxen | 95/5/0.15 | $4.02 \pm 8.00 \times 10^{-3}$ | $1.12 \pm 4.00 \times 10^{-3}$ | $1.69 \pm 4.00 \times 10^{-2}$ |
| *Chiralcel OD* | | | | |
| Carprofen | 90/10/0.15 | $1.88 \pm 4.00 \times 10^{-3}$ | $1.17 \pm 4.00 \times 10^{-3}$ | $1.32 \pm 8.00 \times 10^{-3}$ |
| Fenoprofen | 98/2/0.15 | $2.89 \pm 5.00 \times 10^{-3}$ | $1.12 \pm 2.00 \times 10^{-3}$ | $1.23 \pm 2.00 \times 10^{-3}$ |
| Ibuprofen | 99/1/0.15 | $1.68 \pm 2.00 \times 10^{-3}$ | $1.21 \pm 2.00 \times 10^{-3}$ | $1.70 \pm 5.00 \times 10^{-2}$ |
| Ketoprofen | 98/2/0.40 | $5.07 \pm 3.00 \times 10^{-3}$ | $1.07 \pm 3.00 \times 10^{-3}$ | $0.83 \pm 1.00 \times 10^{-2}$ |
| Naproxen | 95/5/0.15 | $1.65 \pm 2.00 \times 10^{-3}$ | $1.17 \pm 2.00 \times 10^{-3}$ | $1.31 \pm 2.00 \times 10^{-2}$ |

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

* Hexane used for the mobile phase came from a different lot as that used for the analysis of ibuprofen in Fig. 15.

analytes for the unwanted achiral interaction with the accessible free silanol groups of the silica support, thus minimizing this adsorption effect and the resultant peak tailing. In the case of the decrease in the retention, enantioselectivity, and resolution of racemic ibuprofen when TFA concentration was further increased, this may be due to the increase in the polarity of the mobile phase, which in turn increases the solubility of the analyte in the eluent. In addition, the modification of the higher order structure of the CSP, as well as the analyte, may also contribute to this phenomenon. Further discussion of the role of TFA and other acidic modifiers in the enantioseparation of profens on Chiralpak AD and OD is given in Section 5.3.

As shown in Table IV, Fig. 16 and 17, optimized chiral separations of profens on Chiralpak AD and Chiralcel OD using hexane/ethanol/TFA requires mostly less than 10 minutes. Ethanol was used as the mobile phase modifier, instead of the traditional 2-propanol (IPA), because ethanol is more polar and gave a relatively shorter analysis time. In addition, in the study, initial enantioseparations of some profens with IPA were not reproducible. That is, for the first few runs, complete enantioseparations were achieved; then, partial, and finally no separation. Perhaps, more time was required for the conditioning of the chiral columns using hexane/IPA/TFA as the mobile phase.

As shown also in Table IV and Fig. 16, racemic ketoprofen, fenoprofen, and naproxen were completely resolved on Chiralpak AD using hexane/ethanol/TFA. Enantiomers of ibuprofen and carprofen were only partially separated. Furthermore, ketoprofen (Fig. 1), which has the greatest ability among the profens to form more than two hydrogen bonding interactions with Chiralpak AD, showed the highest optimum enantioselectivity. As noted in Chapter III, all profens can form two hydrogen bonding interactions with the CSP: (1) between the acid proton of the carboxyl moiety of profen and the carbonyl oxygen of carbamate moiety of the CSP; and (2) between the carbonyl oxygen of the carboxyl moiety of profen and the amide proton of the carbamate moiety of the CSP. Ketoprofen can form the additional hydrogen bond with the CSP through the keto-oxygen. On the other hand, ibuprofen which can exhibit only two hydrogen bonding interactions with the CSP, has the lowest enantioselectivity. These results strongly suggest that hydrogen bonding interaction is the driving force for chiral recognition.

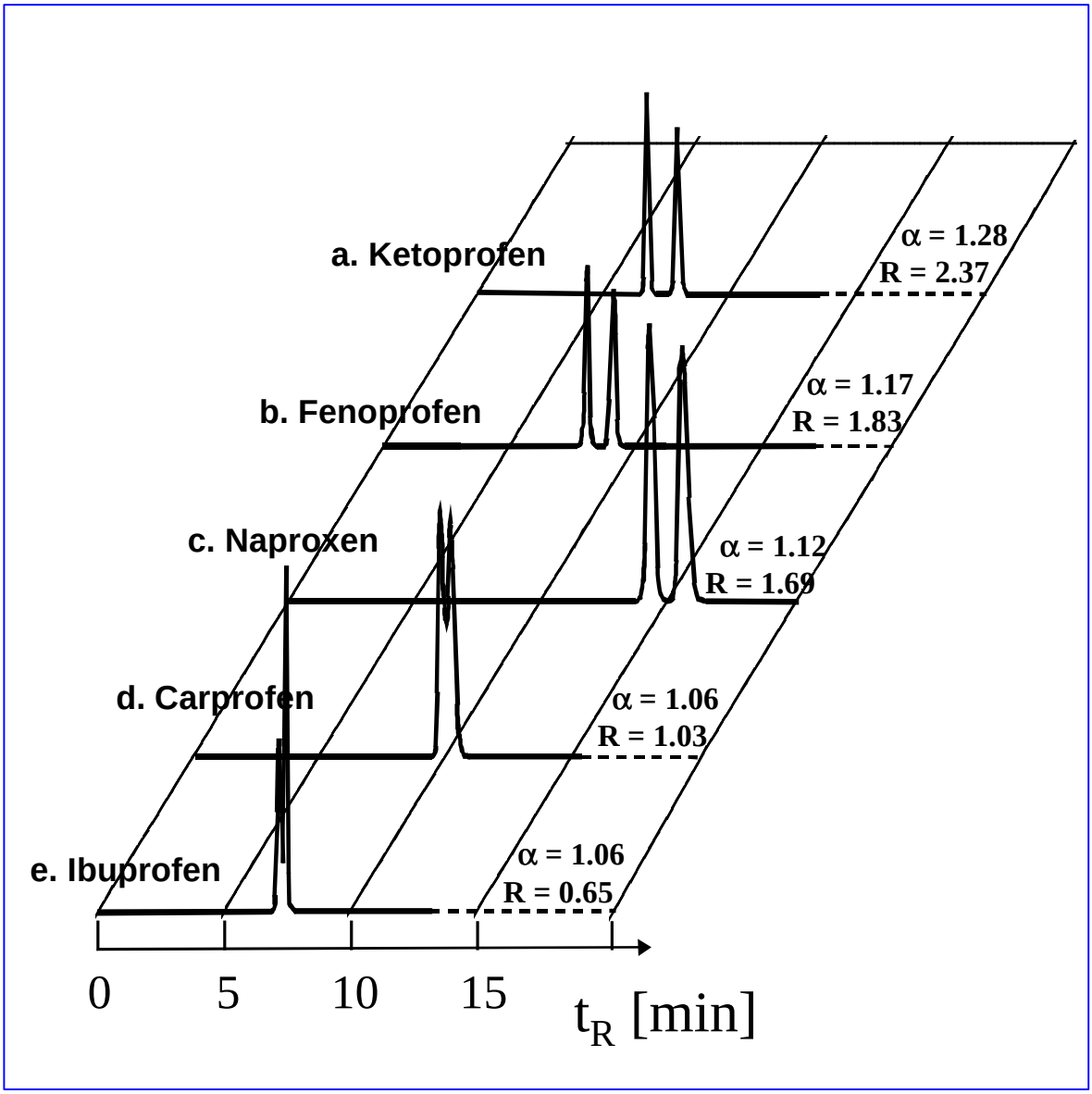


Fig. 16. Chromatograms showing the enantioseparation of profens on Chiralpak AD with hexane/ethanol/TFA at the optimimum conditions: temperature, 25 $^{o}$C; flow rate, 1.0 mL/min; UV detection, 260 nm. Mobile phase: (a) ketoprofen, 80/20/0.15; (b) fenoprofen, 80/20/0.15; (c) naproxen, 95/5/0.15; (d) carprofen, 80/20/0.15; and (e) ibuprofen, 98/2/0.25. Individual enantioselectivity (α) and resolution (R) values are the mean of six replicate injections.

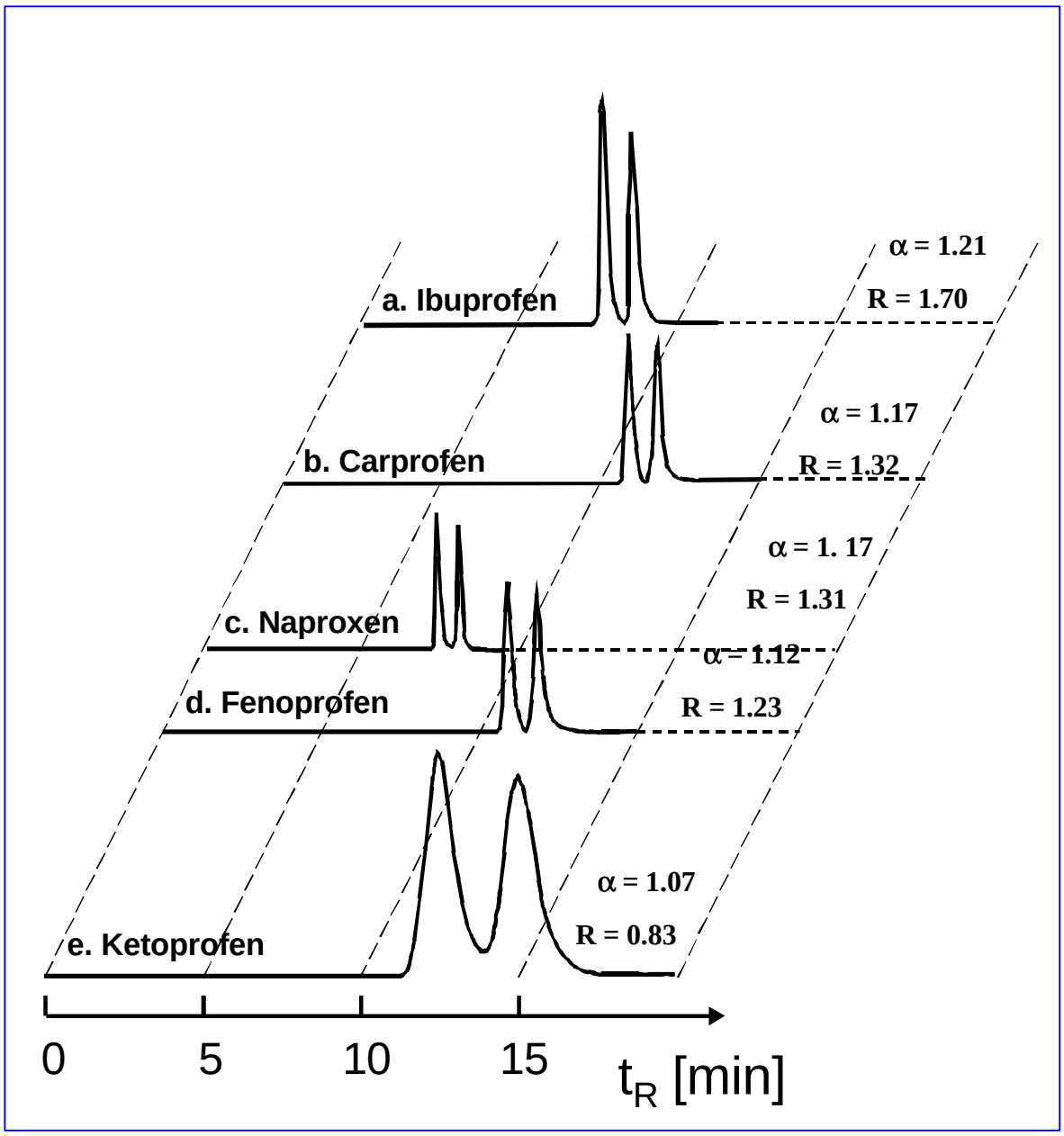


Fig. 17. Chromatograms for the enantioseparation of profens on Chiralcel OD with hexane/ethanol/TFA at the optimimum conditions: temperature, 25 $^{o}$C; flow rate, 1.0 mL/min; UV detection, 260 nm. (a) ibuprofen, 99/1/0.40; (b) carprofen, 90/10/0.15; (c) naproxen, 95/5/0.15; (d) fenoprofen, 98/2/0.15; and (e) ketoprofen, 98/2/0.15. Individual enantioselectivity (α) and resolution (R) values are the mean of six replicate injections.

On Chiralcel OD (Table IV and Figure 17), among the racemic profens, only ibuprofen was completely separated. Ibuprofen, which can make only two hydrogen bonding interactions with the CSP has the highest enantioselectivity. In the case of ketoprofen, which can have more than two hydrogen bonding interactions, has the lowest enantioselectivity and is the least enantioseparated among the profens. It seems that the additional hydrogen bonding interaction is not critical for chiral separation. Furthermore, it appears that the enantioselectivity on Chiralcel OD is dependent on the structure of profen: size, shape, and number of rings (Fig. 1). Ibuprofen, which has only one "free' phenyl moiety, has the highest enantioselectivity. Carprofen and naproxen, which consist of three and two fused rings, respectively, have lower enantioselectivity than ibuprofen. Lastly, ketoprofen and fenoprofen, which have two "free" phenyl moieties, have low enantioselectivities.

The most interesting result was the reversal in the order of optimum enantioselectivity for the profens on Chiralpak AD and Chiralcel OD with hexane/ethanol/TFA. On Chiralpak AD, the decreasing trend of enantioselectivity observed was: ketoprofen, fenoprofen, naproxen, carprofen and ibuprofen (1.28, 1.17, 1.12, 1.06, and 1.06 respectively). Whereas, on Chiralcel OD, the trend of enantioselectivity for the same profens was reversed (1.07, 1.12, 1.17, 1.17, and 1.21 respectively). These results prove that the different arrangement of tris(3,5-dimethylphenylcarbamate)-D-glucose units is responsible for the different enantioseparating abilities of Chiralpak AD and Chiralcel OD. That is, the enantioseparating ability of each CSP is not confined to the interactions of the racemic analyte with the three 3,5-dimethylphenylcarbamate moieties of only one glucose unit but to several. It must be remembered that in Chapter III the structures of Chiralpak AD and Chiralcel OD can be regarded as a left-handed four-fold (4/1) and three-fold (3/2) helices, respectively. The chiral helical cavities of both CSPs are formed by the tris(3,5-dimethylphenylcarbamate)-D-glucose units such that inclusion or steric fit of a racemate may be efficiently discriminated into enantiomers. This reversal of the enantioselectivity order of profens also demonstrated that the enantioseparating abilities of the CSPs are complementary. Moreover, the different enantioselectivities of profens on Chiralpak AD and Chiralcel OD confirm that their higher order structures are different.

## 5.2 Temperature Dependence Studies

The effect of column temperature was explored to gain some insights on the possible chiral recognition mechanism involved in the enantioseparation of profens on Chiralpak AD and Chiralcel OD. Furthermore, the column temperature was also investigated as a possible tool in improving the enantioseparation of profens on Chiralpak AD and Chiralcel OD.

Each racemic profen was chromatographed on Chiralpak AD and Chiralcel OD at 5, 10, 15, 20, and 25$^{o}$C. The mobile phase compositions of hexane/ethanol/TFA used with Chiralpak AD were the following: (a) carprofen, 80/20/0.15; (b) fenoprofen, 80/20/0.15; (c) ibuprofen, 98/2/0.25; (d) ketoprofen, 80/20/0.15; and naproxen, 95/5/0.15. For the enantioseparations on Chiralcel OD, the mobile phase compositions were: (a) carprofen, 90/10/0.15; (b) fenoprofen, 98/2/0.15; (c) ibuprofen, 99/1/0.15; (d) ketoprofen, 98/2/0.40; and (e) naproxen, 95/5/0.15 of hexane/ethanol/TFA.

The influence of column temperature on the retention are summarized by the van't Hoff plots in Fig. 18 and 19. The retention of all enantiomers on both CSPs, expressed by the retention factor (ln $k$), decreased as the column temperature was increased. This result could be attributed to the fact that the analytes, on a molecular level, have smaller adsorption as temperature increases and therefore, a faster migration through the column. Furthermore, the van't Hoff for all profen enantiomers show a linear behavior with regression coefficients ranging from 0.980 to 1.000. It can be inferred that in the temperature range studied, there was no change in the retention mechanism of profen on Chiralpak AD and Chiralcel OD.

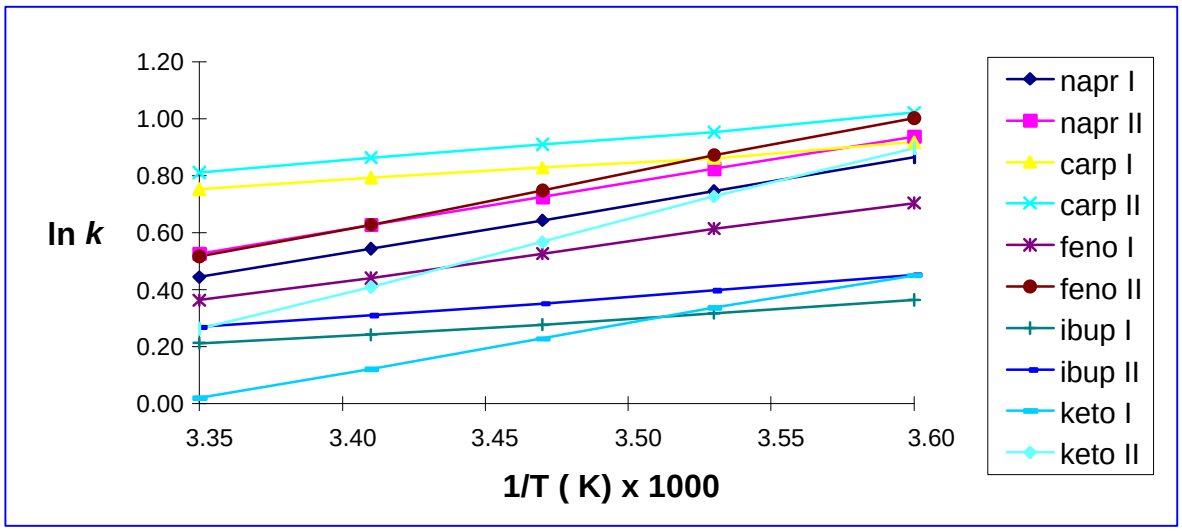


Figure 18. The van't Hoff plots for the enantioseparation of profens on Chiralpak AD. Each data point is the mean of six replicate injections. Linearity of plots with regression coefficients ranged from 0.995 to 1.000, indicates an invariant retention mechanism within the temperature range studied. ***k*** errors ranged from ± 3.48 x $10^{-4}$ to ± 4.8 x $10^{-3}$.

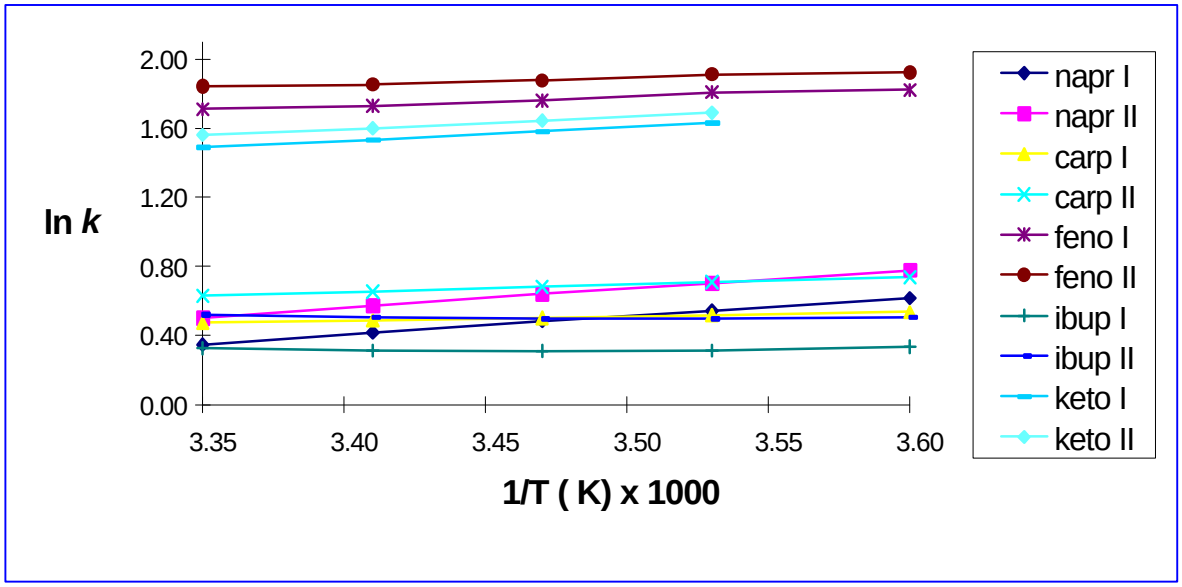


Figure 19. The van't Hoff plots for the enantioseparation of profens on Chiralcel OD. Each data point is the mean of six replicate injections. Regression coefficient of lines ranged from 0.980 to 1.000 which suggest only one retention mechanism within the temperature range. ***k*** errors ranged from ±3.11 x $10^{-4}$ to ± 1.13 x $10^{-3}$.

. For the profens on Chiralpak AD, plots of their enantioselectivities, $\alpha$, against the absolute temperature are shown in Fig. 20. The effect of column temperature is unidirectional. The enantioselectivity of profens increases as the column temperature is decreased, and this is more significant for ketoprofen and fenoprofen. This result may be attributed to a strong force of interaction, such as hydrogen bonding, that is critical for enantioselectivity. The other profens, carprofen, ibuprofen, and naproxen, lowering the column temperature has no marked increase on their enantioselectivities on Chiralpak AD.

The plots for the enantioselectivities of profens on Chiralcel OD are shown in Fig. 21. It shows that the column temperature influences enantioselectivity of profens in both ways depending on their structures. Carprofen and naproxen show an increase in their enantioselectivities as the column temperature is decreased. On the other hand, the enantioselectivity of fenoprofen, ibuprofen, and ketoprofen decreases as the column temperature is decreased. These results also strongly suggests that there are two different mechanisms operating on Chiralcel OD.

Fig. 22 and 23 show the chromatograms for the enantioseparations of fenoprofen on Chiralpak AD and Chiralcel OD from 5 to 25 °C. The separation of racemic fenoprofen on Chiralpak AD appears to be more temperature dependent than that on Chiralcel OD.

The effects of column temperature on the enantioselectivity of profens on Chiralpak AD and Chiralcel OD may be better understood if the thermodynamics for enantioseparation is considered. As discussed in Chapter III, the difference in the free energy of association of the enantiomers with the CSP, $\Delta_{RS}(\Delta G)$, to a first approximation, can be estimated from the enantioselectivity, $\alpha_{RS}$, by

$$\Delta_{RS}(\Delta G) = - RT \ln \alpha_{RS} \qquad (5.2a)$$

The corresponding $\Delta_{RS}\Delta H$ and $\Delta_{RS}\Delta S$ values can be obtained by measuring the $\alpha$ values of the same enantiomeric pair at different temperatures and plotting $R \ln \alpha_{RS}$ versus $1 / T$:

$$R \ln \alpha_{RS} = -\Delta_{RS}\Delta H / T + \Delta_{RS}\Delta S \qquad (5.2b)$$

If $\Delta_{RS}\,\Delta H$ is constant within the temperature range studied, a straight line should be obtained. The slope is $\Delta_{RS}\Delta H$ and the intercept is $\Delta_{RS}\Delta S$.

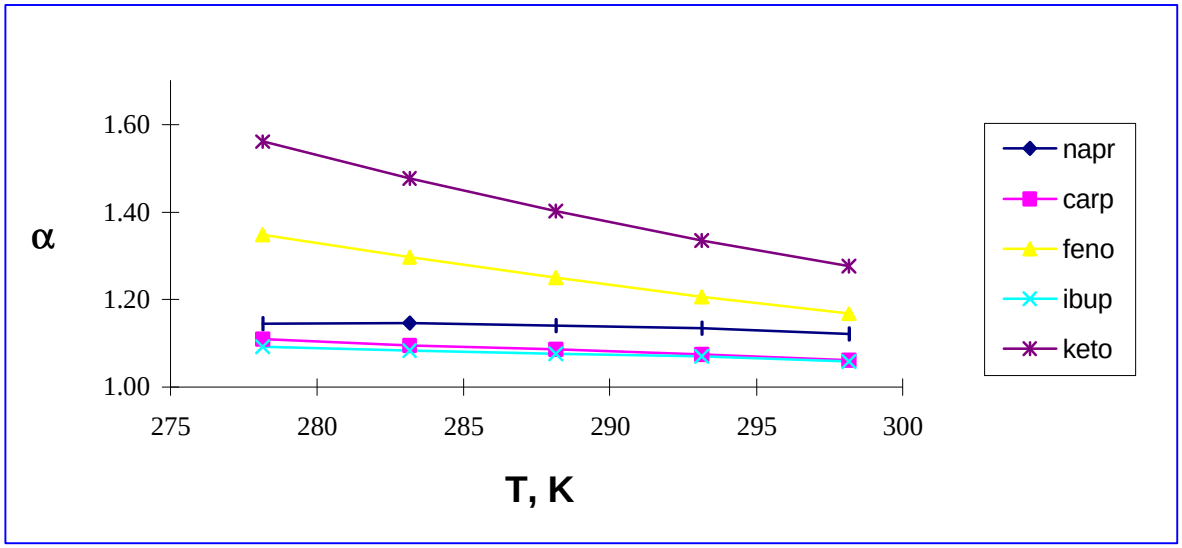


Fig. 20. Plots of the enantioselectivity (α) of profens on Chiralpak AD versus the absolute temperature. A unidirectional temperature dependence of α is shown. Each data point is the mean of six replicate injections. The relative standard deviations of α ranged from 0.16 to 0.47 %.

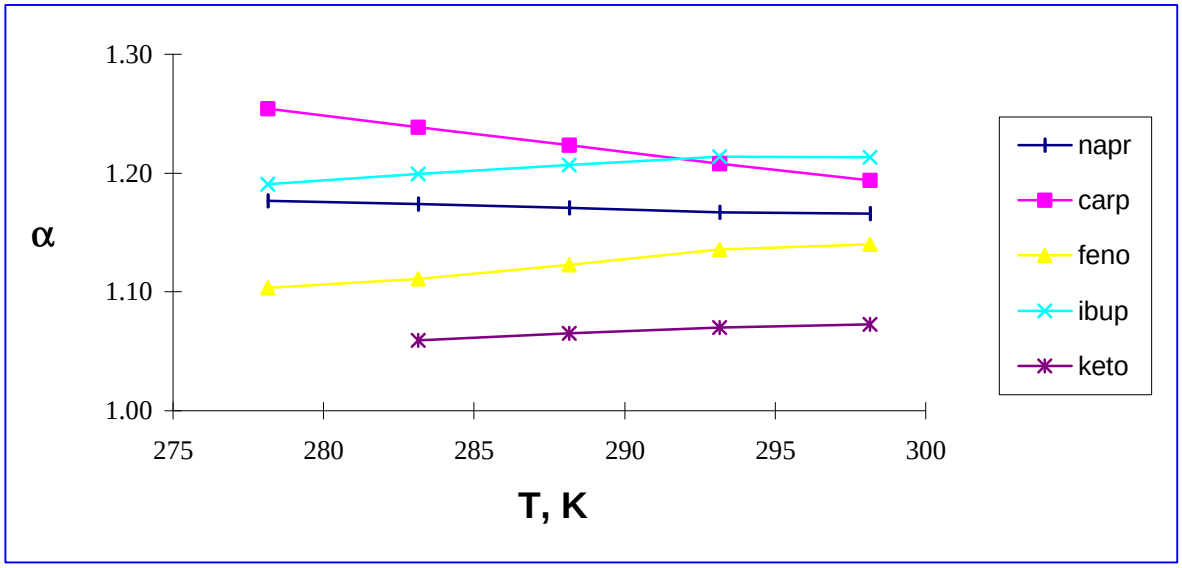


Fig. 21. Plots of the enantioselectivity of profens on Chiralcel OD versus the absolute temperature. Each data point is the mean of six replicate injections. Each data point is the mean of six replicate injections. The relative standard deviations of α ranged from 0.17 to 0.34 %.

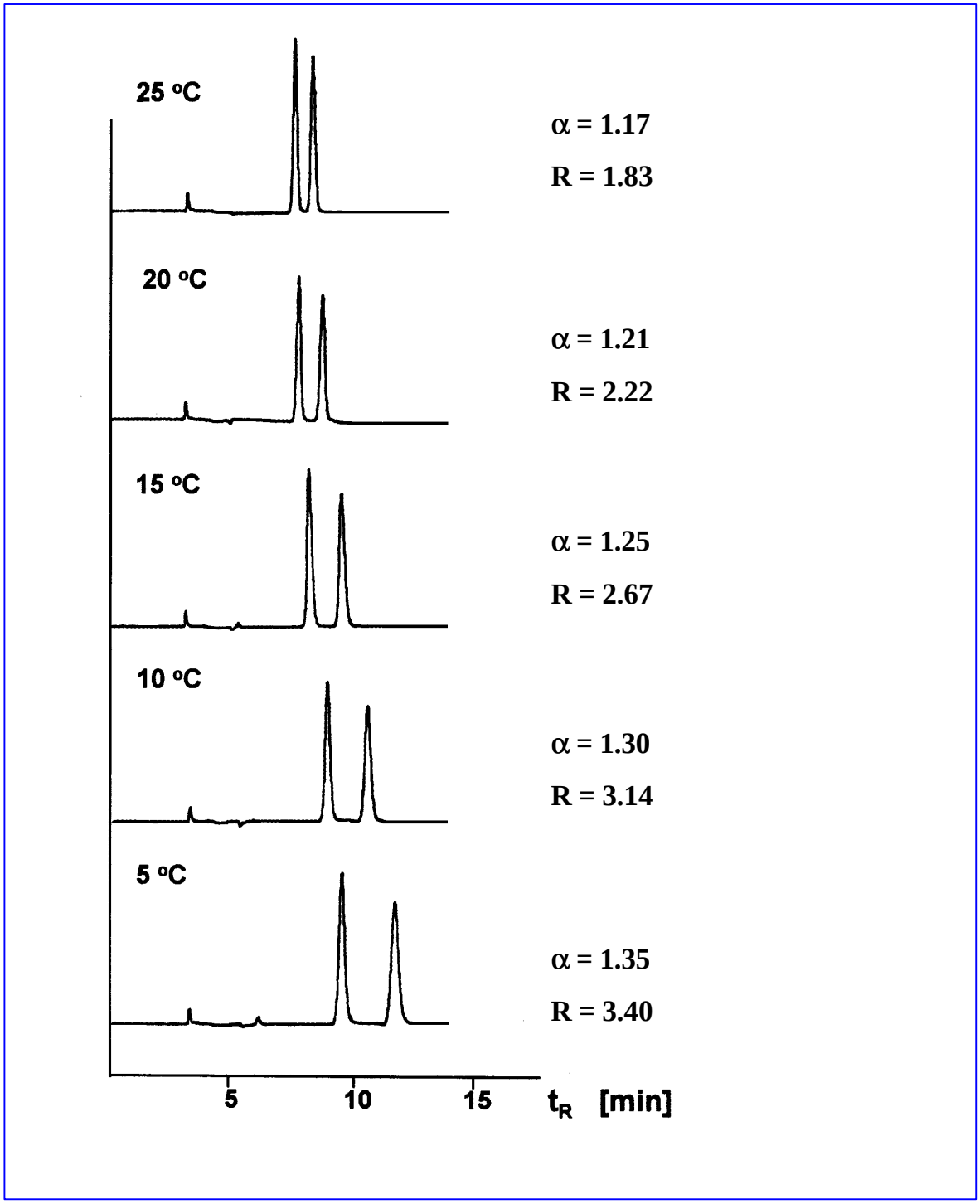


Figure 22. Chromatograms showing the temperature dependence of the enantioseparation of fenoprofen on Chiralpak AD with 80/20/0.15 of hexane/ethanol/TFA, 1.0 mL/min, and UV detection of 260 nm. Individual enantioselectivity (α) and resolution (R) values are the mean of six replicate injections.

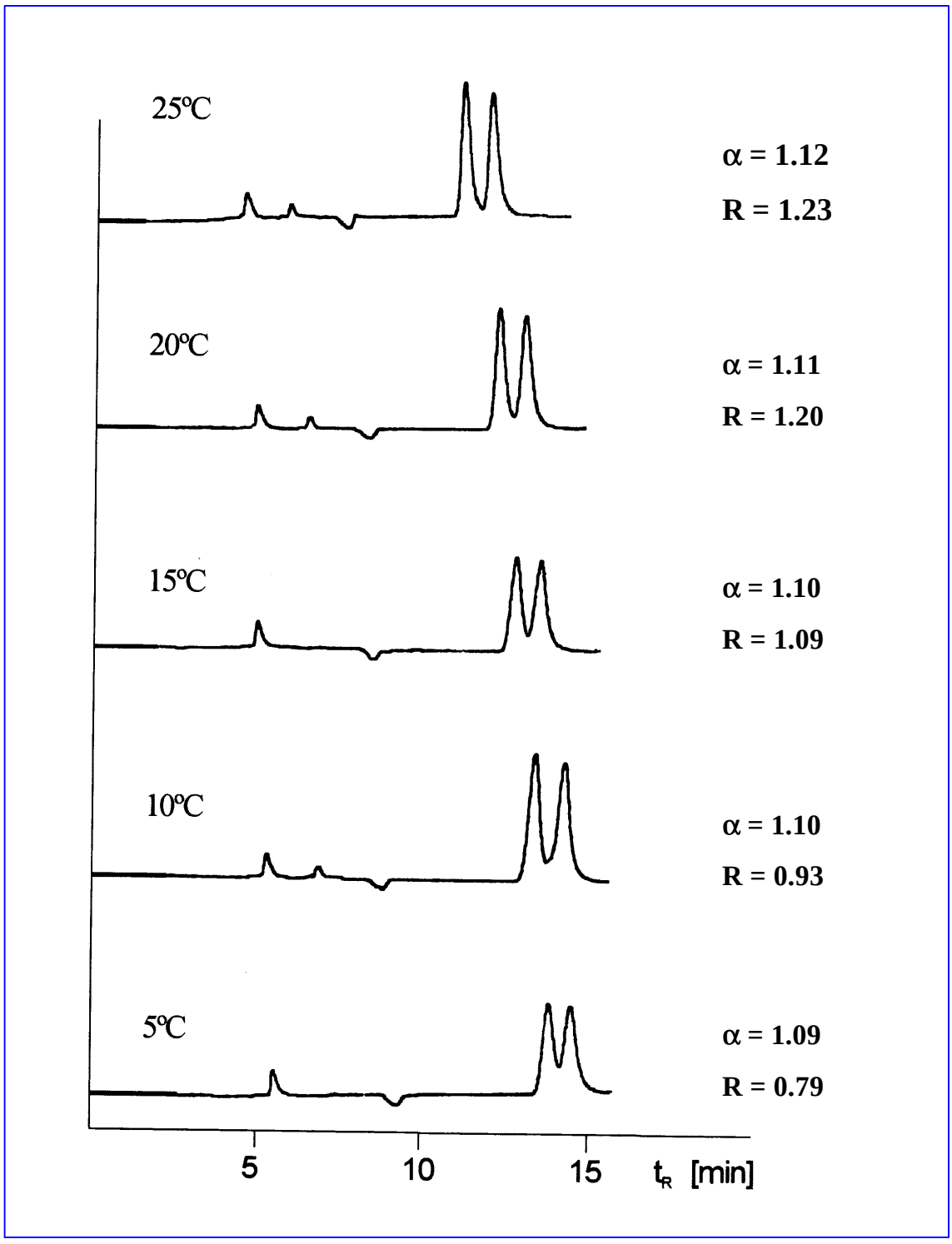


Figure 23. Chromatograms showing the temperature dependence of the enantioseparation of fenoprofen on Chiralcel OD with 98/2/0.15 hexane/ethanol/TFA, 1.0 mL/min, and UV detection of 260 nm. Individual enantioselectivity (**α**) and resolution (**R**) values represent six replicate injections.

Calculated values for the thermodynamic parameters, $\Delta_{RS}\,\Delta H$, $\Delta_{RS}\,\Delta S$, and $\Delta_{RS}\,\Delta G$, from the plots of $\boldsymbol{R \ln \alpha_{RS}}$ versus **1/T** (Fig. 24 and 25) are given in Table V. The $\Delta_{RS}\,\Delta H$ values for the profens on Chiralpak AD are all negative indicating an enthalpy controlled enantioseparations. Interestingly, on Chiralpak OD, only carprofen and naproxen have negative $\Delta_{RS}\,\Delta H$ values. Fenoprofen, ibuprofen, and ketoprofen have positive $\Delta_{RS}\,\Delta H$ values suggesting entropy controlled enantioseparations.

Usually, as most HPLC separations are enthalpy controlled. All the racemic profens have negative $\Delta_{RS}\,\Delta H$ values showed an increase in enantioselectivity, thus an increase in chiral resolution, when the temperature was decreased. Enthalpy changes in this context arise mainly due to heats of adsorption during retention, and as consequence of partial bonding to the selector, since for two enantiomers solvation enthalpies must be identical. $\Delta_{RS}\,\Delta H$ determines the slope of the *ln k vs. **1/T*** graphs. When two enantiomers show large $\Delta_{RS}\,\Delta H$ values, chiral recognition is considered to be very temperature dependent. In Table V, the $\Delta_{RS}\,\Delta H$ values for ketoprofen on Chiralpak AD, for example, is very large (-1663 cal/mol) and the corresponding $\Delta_{RS}\,\Delta S$ value (-1520 cal/mol) is smaller at 25 °C. One interpretation is that the chiral discrimination involves hydrogen bonding, which is a very temperature dependent phenomenon. For ketoprofen on Chiralpak AD, chiral recognition may depend critically on the hydrogen bond formed between the keto oxygen and the amide proton of the carbamate moiety of the CSP. As discussed previously the two other hydrogen bonding interactions between the carbonyl oxygen and the acidic proton of the carboxyl group of the profens and the amide proton and carbonyl oxygen of the carbamate moiety of the CSP (Fig. 26) are also important in chiral recognition. In addition, insertion of a profen into chiral cavities , as well as π-π interactions between the phenyl groups of the profens and the 3,5-dimethylphenylcarbamate moiety of the CSP and dipole-dipole interactions, may also contribute to chiral discrimination.

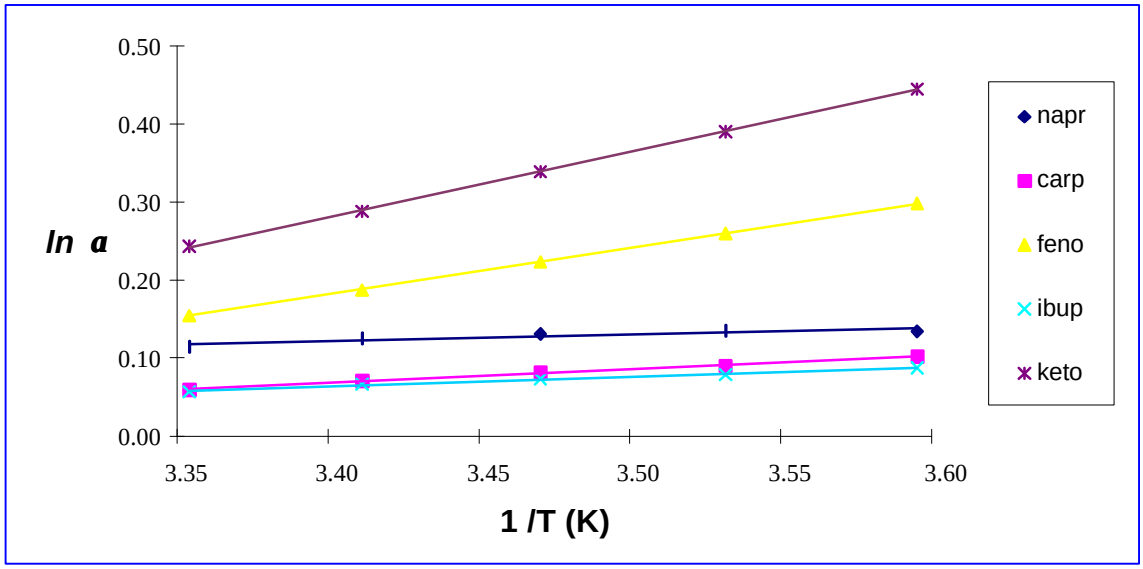


Fig. 24. The plots of ***ln*** **α** vs. **1/*T*** for the enthalpy controlled enantioseparation of profens on Chiralpak AD. The thermodynamic parameters of $\Delta_{RS}\,\Delta H$ and $\Delta_{RS}\,\Delta S$ are represented by the slope and the intercept, respectively. Each data point is the mean of six replicate injections.

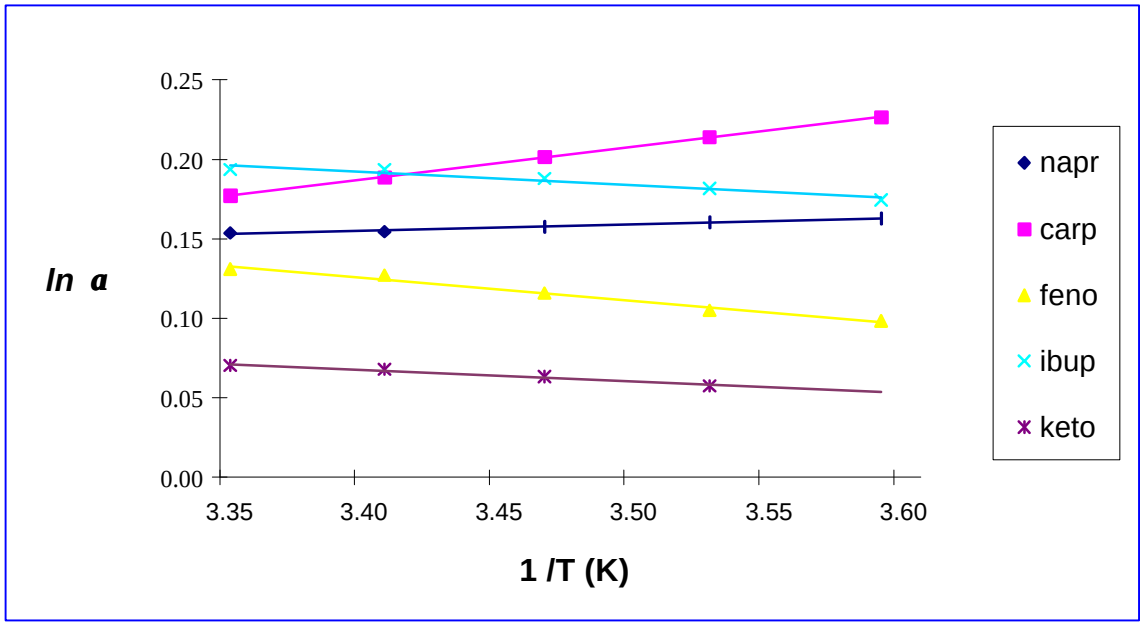


Fig. 25. The plots of ***ln*** **α** vs. **1/*T*** showing both enthalpy and entropy controlled enantioseparation of profens on Chiralcel OD. Each data point is the mean of six chromatographic injections.

**Table V. Measured thermodynamic parameters on Chiralpak AD and Chiralcel OD.**

| Racemates | Temp. Range (°C) | $-\Delta_{RS}\Delta H/R$ K | $\Delta_{RS}\Delta S/R$ | $\Delta_{RS}\Delta H$ cal/mol | $T\Delta_{RS}\Delta S$ (298 K) cal/mol | $\Delta_{RS}\Delta G$ (298 K) cal/mol | $T_{iso}$ (°C) |
|---|---|---|---|---|---|---|---|
| *Chiralpak AD* | | | | | | | |
| Ketoprofen | 5 → 25 | -837 | -2.6 | -1663 | -1520 | -143 | 53 |
| Fenoprofen | 5 → 25 | -595 | -1.8 | -1183 | -1091 | -92 | 50 |
| Naproxen | 5 → 25 | -83 | -0.2 | -165 | -95 | -70 | 245 |
| Carprofen | 5 → 25 | -176 | -0.5 | -349 | -313 | -36 | 59 |
| Ibuprofen | 5 → 25 | -121 | -0.3 | -240 | -206 | -35 | 75 |
| *Chiralcel OD* | | | | | | | |
| Ibuprofen | 5 → 25 | +86 | +0.5 | +171 | +287 | -116 | -96 |
| Carprofen | 5 → 25 | -190 | -0.5 | -377 | -283 | -93 | 123 |
| Naproxen | 5 → 25 | -40 | -0.02 | -80 | -11 | -68 | 1833 |
| Fenoprofen | 5 → 25 | +147 | +0.6 | +292 | +359 | -67 | -31 |
| Ketoprofen | 10 → 25 | +73 | +0.3 | +145 | +187 | -42 | -49 |

Measured values were taken from plots of of *ln* α *vs*. 1/*T* (Fig. 24 and 25) where $\Delta\Delta H/R$ and $\Delta\Delta S/R$, are the slope and intercept, respectively. Average of six runs, 25°C, flow rate 1.0 mL/min. Mobile phase: hexane/ethanol/TFA, the same composition as in Table IV. Enthalpy error range: ± 0.8% to 30% (10 to 70 cal/mol), 95% C.I.

In the case of fenoprofen, ibuprofen, and ketoprofen on Chiralcel OD, the $\Delta_{RS}\,\Delta H$ values are positive and larger than the corresponding $\Delta_{RS}\,\Delta S$ values at a given temperature (Table V). Thus, the temperature dependence of the chiral separation is dominantly entropy controlled. Therefore, lowering the column temperature both decreases enantioselectivity and chiral resolution. This is better understood by considering the $\Delta_{RS}\,\Delta S$ data in Table V in more detail. For example, at 25 °C, ibuprofen on Chiralcel OD shows values of +170.8 cal and +287.2 cal for $\Delta_{RS}\,\Delta H$ and $\Delta_{RS}\,\Delta S$, respectively. The difference in free energy, $\Delta_{RS}\,(\Delta G)$ = -116.4 cal/mol, leads to a chiral discrimination that is mainly driven by a drastic increase in entropy with an increase in temperature. This may be interpreted in terms of the steric fit of enantiomers to the chiral cavity of Chiralcel OD, which is critical for chiral discrimination. Ibuprofen, fenoprofen, and ketoprofen have at least one "free phenyl ring". Entropy is a measure of molecular order, hence it plays a role when the stationary phase has the preference for a certain size and shape of analyte

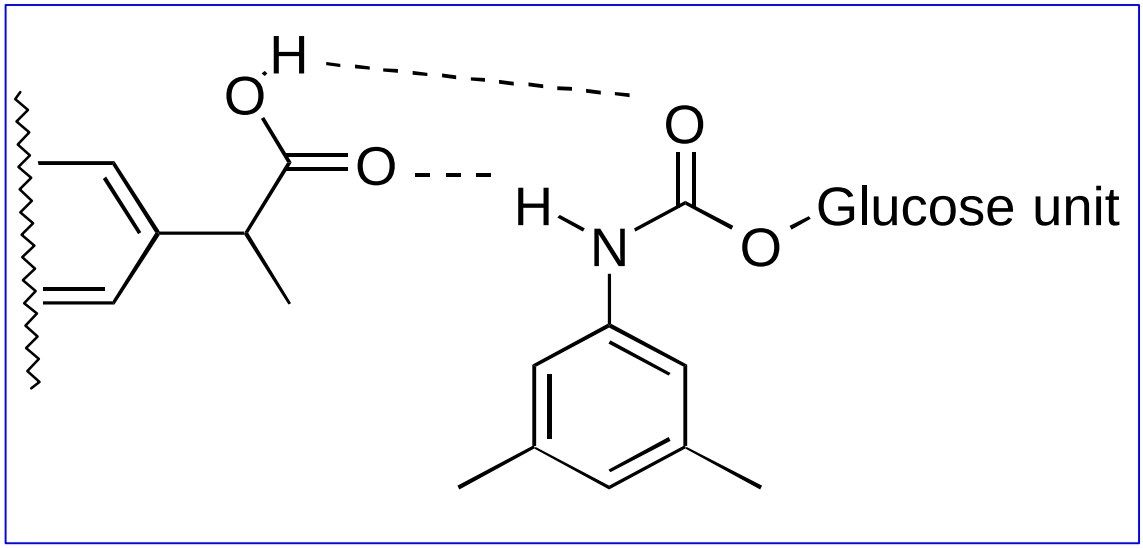


Fig. 26. Hydrogen bonding between the hydrogen and carbonyl oxygen of the carboxyl moiety of profen with the amide proton and carbonyl oxygen of the carbamate moiety of the CSP.

molecule. For naproxen and carprofen which contain fused rings and appear to be exhibiting enthalpy controlled mechanism, steric fit to the chiral cavity may not be critical for chiral discrimination.

The isoenantioselective temperatures for each profens on Chiralpak AD and Chiralcel OD were calculated and are shown in Table V. However, chromatographic runs at the isoenantioselective temperatures were not performed due to the working temperature range restrictions.

Considering the $\Delta_{RS}$ ($\Delta G$) values at 25 °C (Table V) that were calculated from the plots of *ln* ***a*** versus *1/T*, the decreasing order on Chiralpak AD is ketoprofen, fenoprofen, naproxen, carprofen, and ibuprofen (-143.1, -91.6, -70.0, -35.7, and -34.6 cal/mol, respectively). This decreasing trend also holds true for the optimized enantioselectivity of the same profens chromatographed at 25 °C (1.28, 1.17, 1.13, 1.07, and 1.07, respectively; see Table 5.1). On Chiralcel OD, there is the reversal of the trend in $\Delta_{RS}$ ($\Delta G$): ibuprofen, carprofen, naproxen, fenoprofen, and ketoprofen (116.4, -93.3, -68.4, -67.4, and 42.1 cal/mol, respectively). The reversal of the trend for $\Delta_{RS}$ ($\Delta G$) is the same for the enantioselectivity of the same profens chromatographed at 25°C on Chiralcel OD (1.21, 1.17, 1.17, 1.117, 1.07 cal/mol; respectively, see Table V). This could be expected since from Equation 5.2a, $\Delta_{RS}$ ($\Delta G$) is directly related to $\alpha_{RS}$.

The influence of column temperature on the enantiomer resolution (**R**) of profens on Chiralpak AD and Chiralcel OD are presented in Fig. 27 and 28. Resolution is a complicated function of the retention factor (***k***), selectivity ($\alpha$), and column efficiency (**N**):

$$\mathbf{R = (k / k + 1) (\alpha - 1 / \alpha) (N / 16) \exp 0.5} \qquad (5.2c)$$

The results of the study showed that the resolution of all racemic profens on Chiralpak AD were enhanced (Fig. 22 and 27), accompanied by an increase in retention and enantioselectivity, when the temperature was decreased (Fig. 18 and 20). Whereas on Chiralcel OD, the enantiomer resolution was influenced both ways by the column temperature. For the entropy controlled enantioseparation, i.e., characterized by a decrease in retention and an increase in enantioselectivity when the temperature is increased, this gave a high enantiomer resolution at high column temperature. This phenomenon is exemplified by racemic fenoprofen on Chiralcel OD (Fig. 23 and 28). For

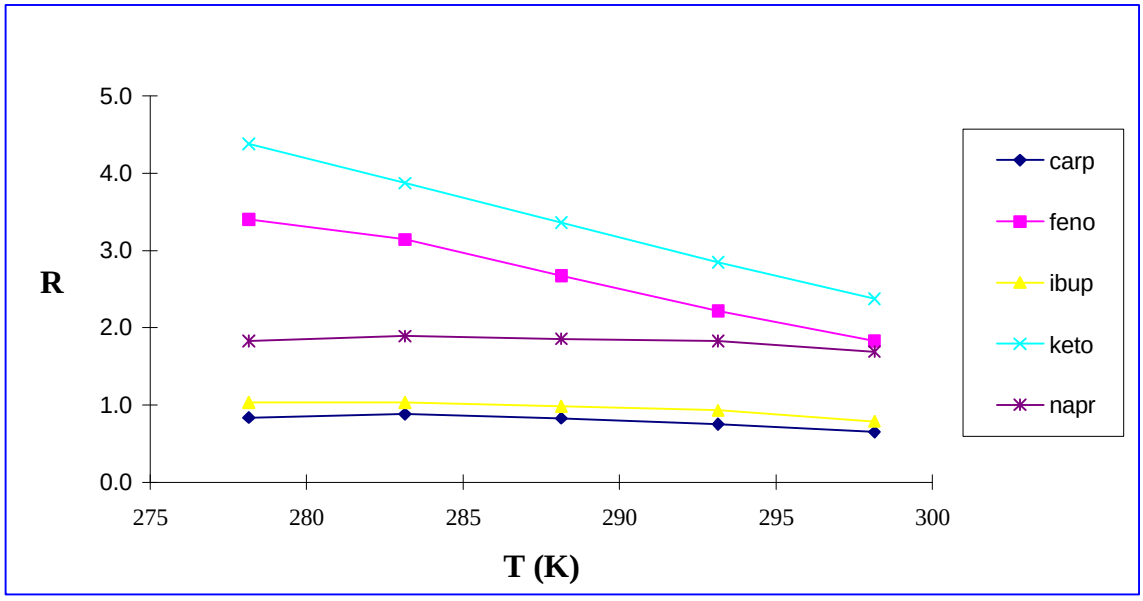


Figure 27. The plots of resolution (**R**) versus the absolute column temperature for racemic profens on Chiralpak AD is shown. **R** is enhanced when the temperature is decreased. Each cluster of points represents six replicate injections.

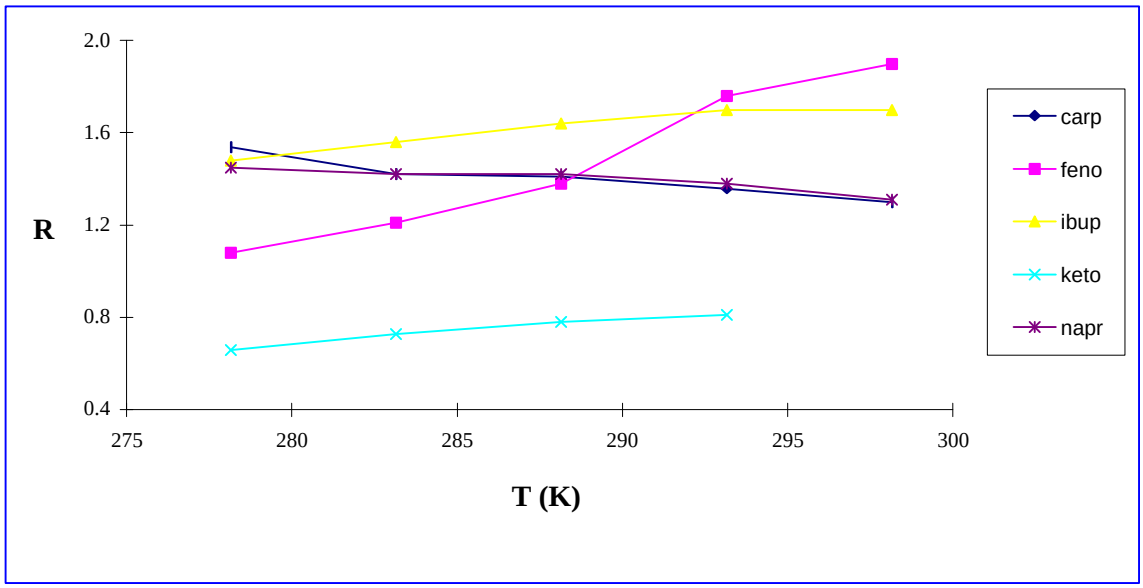


Figure 28. Resolution (**R**) of racemic profens on Chiralcel OD versus the absolute column temperature is plotted. A bidirectional dependence of **R** on column temperature is shown. Each data is the mean of six replicate injections.

the enthalpy controlled enantioseparation on Chiralcel OD, e.g., carprofen and naproxen, this was characterized by an increase in resolution associated with an increase in both retention and enantioselectivity (Fig. 28, 19, and 21).

In summary, the influence of temperature on the retention of profens on Chiralpak AD and Chiralcel OD is unidirectional, i.e., a decrease in column temperature increases retention. The effect of temperature on the enantioselectivity of profens on Chiralpak AD is also unidirectional. Enantioselectivity increased when the column temperature was decreased. In addition, the enantiomer resolution was enhanced. The enantioseparations were very temperature dependent and said to be enthalpy controlled, characterized by large values $\Delta_{RS}\Delta H$ and small $\Delta_{RS}\Delta S$. In practical terms, the optimization of the enantioseparation of profens on Chiralpak AD can be done by lowering the column temperature. On Chiralcel OD, on the other hand, the temperature dependence of enantioseparation of profens is bidirectional. An increase in column temperature may either increase or decrease enantioselectivity depending on the structure of the profen. For the entropy controlled enantioseparation, an increase in column temperature improves enantioselectivity, thus, the enantioresolution. This bidirectional dependence of temperature further demonstrated that there are at least two chiral recognition mechanisms operating on the Chiralcel OD CSP.