5.3. MobilePhase Studies (Polar Modifiers)

5.3.1 Acidic Mobile Phase Modifiers

Previous discussion of the enantioseparation of profens on Chiralpak AD and Chiralcel OD explained the need of TFA for resolution. Without TFA not a single pair of enantiomers was separated. This study investigated the influence of various acidic mobile phase modifiers on retention and enantioselectivity of profens on Chiralpak AD and Chiralcel OD. All chiral separations were performed at constant molar concentration of ethanol in hexane at 25°C and at constant concentrations of the acidic modifiers. Doing this was to insure that the number of modifier molecules competing for sites on the CSP were equal in each experiment. For example, 80/20/0.15 of hexane/EtOH/acidic-modifier is 3.43 M EtOH in hexane and 1.95×10^{-6} M with respect to the acidic modifier. The acidic mobile phase modifiers used were acetic acid (HOAc), heptaflurobutyric acid (HFBA), and TFA. The choice of the acidic modifiers was limited to those acids that are compatible with the CSP. The structures of the acidic mobile phase modifiers and ionization constants are shown in Table VI.

The acidic mobile phase modifiers may compete with the profens for the chiral active sites of Chiralpak AD and Chiralcel OD. All acidic modifiers have the ability to form two hydrogen bonds, by virtue of the carboxylic acid functionality, with the carbamate moiety of the CSP. To further investigate the effect of acidic mobile phase modifiers on retention and enantioselectivity, three methyl esters of fenoprofen, ibuprofen, and ketoprofen (Fig. 29) and another profen , flurbiprofen were included in the study. The methyl esters of profens, by virtue of the carboxylate functionality, are capable of forming only one hydrogen bond with the carbamate moiety of the CSP. The specific interactions between the acid modifiers and CSP are discussed at the end of this section.

Table VI. Structures and acid ionization constants of the acidic mobile phase modifiers used in the study.

Organic Acid	Structure	Acid Ionization Constant (pKa)
Acetic acid (HOAc)	CH_3COOH	1.8×10^{-5} (4.7)
Trifluoroacetic acid (TFA)	CF_3COOH	5.0×10^{-1} (0.3)
Heptafluorobutyric acid (HFBA)	$CF_3CF_2CF_2COOH$	4.0×10^{-1} (0.4)

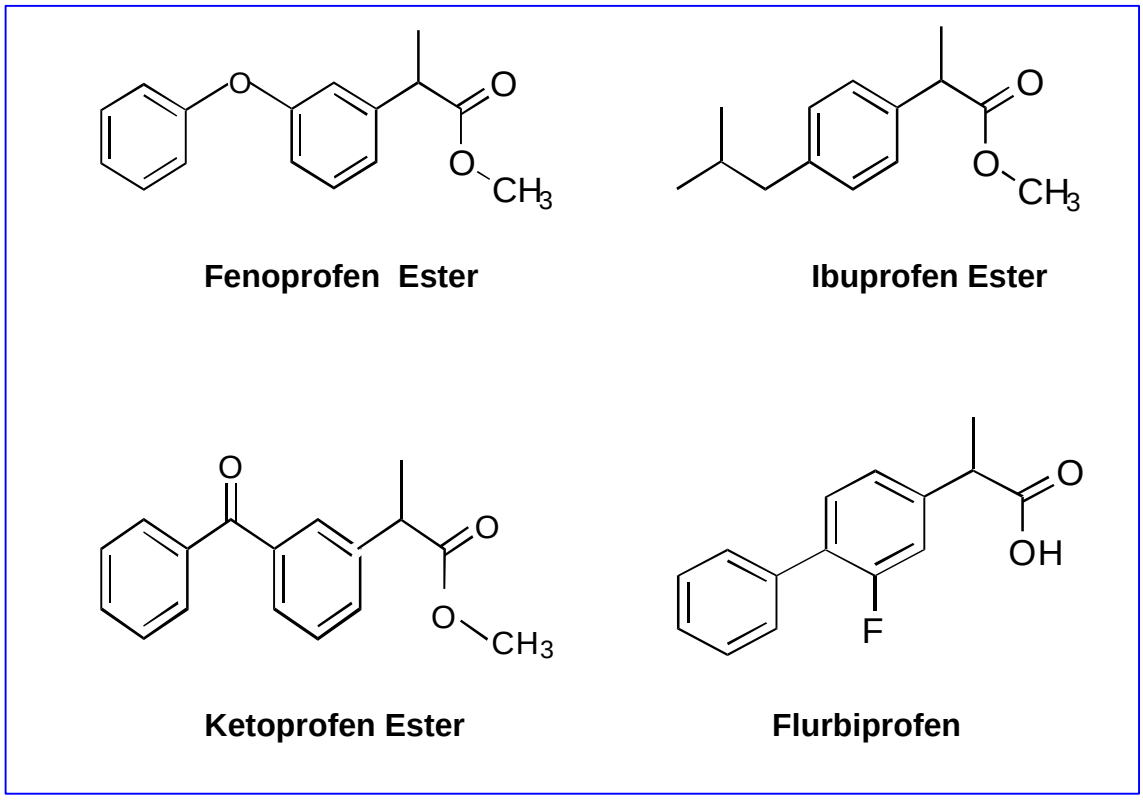


Figure 29. Additional probe analytes used in the study.

As shown in Table 5.4 and Fig. 5.15, the presence of the acidic organic modifier in the enantioseparation of profens on Chiralpak AD results to a significant decrease of retention which is represented by the capacity factor of the second eluting enantiomer, k_2. The decreases in k_2 following addition of HAc, HFBA, and TFA to ethanol in hexane average around 87, 152, and 150 %, respectively. When the acidic modifier is changed from the weaker acetic acid (pKa = 4.7) to stronger acids of HFBA (pKa = 0.4) and TFA (pKa = 0.3), the decrease in k_2 average about 31 and 30%, respectively. Furthermore, the conversion of the profen to its corresponding methyl ester, results to a drastic decrease in k_2 (Table 5.5). The decrease in k_2 following the conversion of profen to its methyl ester averages about 384%. However, there is no significant change in k_2 for all the profen and profen esters when HFBA is changed to TFA as the acidic modifier, the average is about 5%.

Considering the change in α, following addition of HOAc, HFBA, and TFA to the mobile phase, there is an increase that averages about 18, 30, 29%, respectively (Table VII). Changing the acidic modifier from HOAc to HFBA or TFA, increases α around 9%. There is no marked effect on α when HFBA is changed to TFA, overall average is about 0.7% . In addition, among all the profen methyl esters analyzed, only the enantiomers of ketoprofen ester is separated but partially. There was significant effect on the enantioselectivity of ketoprofen methyl esters when different mobile phase compositions of hexane/ethanol/acidic-modifier were used, even when the overall change of retention is 207%. The overall change in enantioselectivity averages around 2% (Table VIII).

Enantiomer resolution of profens was facilitated when HOAc, HFBA, or TFA was added to the mobile phase. There was a significant improvement in resolution when HOAc was changed to either HFBA or TFA. The overall increase average around 89%. When the acidic modifier was changed from HFBA to TFA, except for carprofen, there was an overall increase of about 8%.

The influence of the acidic mobile phase modifiers on the enantioseparation of ketoprofen and its methyl ester on Chiralpak AD is shown in Fig. 30. Racemic ketoprofen is best separated when TFA was used as the acidic mobile phase modifier.

Table VII. Summary of the measured chromatographic parameters for the enantioseparation of profens on Chiralpak AD using different acidic mobile phase modifiers.

Profen	Acidic Mobile Phase Modifier	k_2[a]	Enantio-selectivity α	Resolution **R**
Carprofen	None	7.71[b]	1.00[b]	0.00[b]
(3.43 M EtOH in	HOAc	$3.30 \pm 1.02 \times 10^{-2}$	$1.00 \pm 1.02 \times 10^{-2}$	0.00
hexane with 1.95×10^{-6}	HFBA	$2.09 \pm 3.51 \times 10^{-3}$	$1.07 \pm 1.87 \times 10^{-3}$	$0.69 \pm 1.99 \times 10^{-2}$
moles organic acid)	TFA	$2.08 \pm 8.70 \times 10^{-4}$	$1.06 \pm 6.18 \times 10^{-4}$	$0.67 \pm 7.30 \times 10^{-3}$
Fenoprofen	None	2.91[b]	1.00[b]	0.00[b]
(0.43 M EtOH in	HOAc	$2.83 \pm 5.92 \times 10^{-3}$	$1.10 \pm 3.53\ 10^{-3}$	$1.20 \pm 1.00 \times 10^{-2}$
hexane with 1.95×10^{-6}	HFBA	$2.63 \pm 7.74 \times 10^{-4}$	$1.11 \pm 8.48 \times 10^{-4}$	$1.42 \pm 1.84 \times 10^{-3}$
moles organic acid)	TFA	$2.69 \pm 8.62 \times 10^{-4}$	$1.13 \pm 3.79 \times 10^{-4}$	$1.01 \pm 7.93 \times 10^{-3}$
Flurbiprofen	None	2.80[b]	1.00[b]	0.00[b]
(0.43 M EtOH in	HOAc	$4.15 \pm 2.41 \times 10^{-2}$	$1.44 \pm 3.27 \times 10^{-2}$	$5.82 \pm 1.62 \times 10^{-1}$
hexane with 1.95×10^{-6}	HFBA	$3.84 \pm 2.03 \times 10^{-3}$	$1.70 \pm 9.87 \times 10^{-4}$	$7.44 \pm 2.31 \times 10^{-2}$
moles organic acid)	TFA	$3.81 \pm 2.71 \times 10^{-3}$	$1.70 \pm 1.40 \times 10^{-3}$	$7.55 \pm 3.12 \times 10^{-2}$
Ketoprofen	None	3.52[b]	1.00[b]	0.00[b]
(0.43 M EtOH in	HOAc	$1.82 \pm 1.44 \times 10^{-2}$	$1.16 \pm 1.40 \times 10^{-2}$	$0.74 \pm 4.00 \times 10^{-2}$
hexane with 1.95×10^{-6}	HFBA	$1.21 \pm 3.83 \times 10^{-4}$	$1.26 \pm 9.30 \times 10^{-4}$	$2.21 \pm 9.21 \times 10^{-3}$
moles organic acid)	TFA	$1.23 \pm 3.31 \times 10^{-3}$	$1.27 \pm 1.43 \times 10^{-3}$	$2.35 \pm 1.33 \times 10^{-2}$

[a]Retention factor of the second eluted enantiomer.

Average of six runs 25°C, flow rate 1.0 mL/min. Exceptions are [b] which are average of two runs.

Table VIII. Influence of acidic mobile phase modifiers on retention and enantioselectivity of profen methyl esters on Chiralpak AD.

Profen	Acidic Mobile Phase Modifier	k_2*	Enantio-selectivity α	Resolution **R**
Fenoprofen Ester (0.43 M EtOH in hexane with 1.95 x 10^{-6} moles acid)	HOAc	0.39 ± 4.65 x 10^{-4}	1.00 ± 1.70 x 10^{-3}	0.00
	HFBA	0.39 ± 2.83 x 10^{-4}	1.00 ± 9.39 x 10^{-4}	0.00
	TFA	0.41 ± 3.22 x 10^{-4}	1.00 ± 1.12 x 10^{-3}	0.00
Ibuprofen Ester (0.34 M EtOH in hexane with 1.95 x 10^{-6} moles acid)	HOAc	0.12 ± 4.00 x 10^{-3}	1.00 ± 5.00 x 10^{-3}	0.00
	HFBA	0.12 ± 4.00 x 10^{-4}	1.00 ± 5.00 x 10^{-3}	0.00
	TFA	0.13 ± 7.00 x 10^{-4}	1.00 ± 8.00 x 10^{-3}	0.00
Ketoprofen Ester				
a. (0.34 M EtOH in hexane with 1.95 x 10^{-6} moles acid)	HOAc	2.00 ± 1.02 x 10^{-3}	1.04 ± 7.28 x 10^{-4}	0.55 ± 1.33 x 10^{-2}
	HFBA	2.04 ± 3.42 x 10^{-3}	1.03 ± 2.48 x 10^{-3}	0.50 ± 4.20 x 10^{-2}
	TFA	2.11 ± 1.80 x 10^{-3}	1.04 ± 1.24 x 10^{-3}	0.57 ± 2.09 x 10^{-2}
b. (0.43 M EtOH in hexane with 1.95 x 10^{-6} moles acid)	HFBA	0.67 ± 5.00 x 10^{-4}	1.06 ± 2.00 x 10^{-3}	0.50 ± 2.00 x 10^{-2}
	TFA	0.68 ± 3.00 x 10^{-4}	1.06 ± 1.00 x 10^{-3}	0.50 ± 1.00 x 10^{-2}

*Retention factor of the second eluted enantiomer.

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

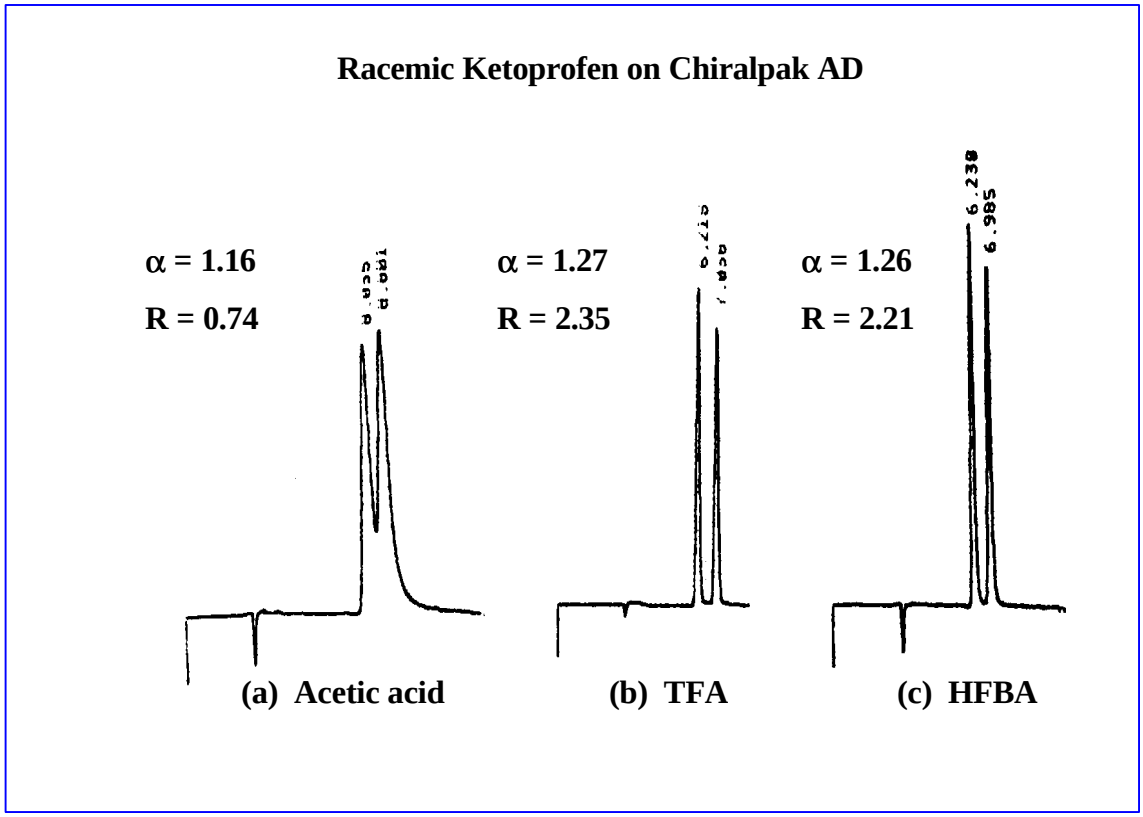
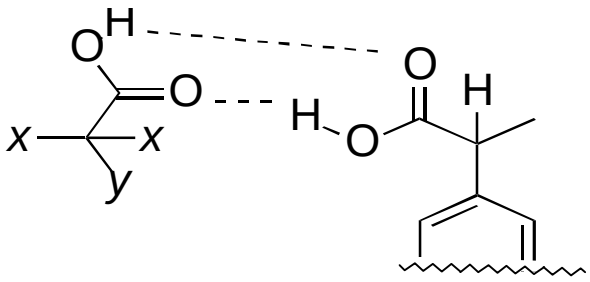


Figure 30. Chromatograms showing the influence of acidic mobile phase modifiers on the enantioseparation of ketoprofen on Chiralpak AD. Chromatographic conditions: mobile phase composition, 3.43 M EtOH in hexane with 1.95 x 10^{-6} moles of acidic modifier; flow rate, 1.0 mL/min; UV detection: 260 nm, temperature: 25°C. Individual enantioselectivity (**a**) and resolution (**R**) values are the mean of six replicate injections.

The steady decrease in k_2 and simultaneous increase in α strongly indicates that there is change in the mechanism of the racemic analyte interaction with the CSP before and after the addition of the acidic mobile phase modifier. The acidic modifiers, by virtue of the carboxylic moiety, are capable of forming a complex with the profens or CSP through hydrogen bonding. The association of the acidic modifier and profen is through two hydrogen bonding between the carbonyl oxygen and hydroxyl proton of two carboxyl moities (Fig. 31a). With the CSP, the acidic modifier also forms two hydrogen bonds, i.e., between the carbonyl oxygen and hydroxyl proton of the carboxyl group of the acid and the amide proton and carbonyl oxygen of the carbamate moiety of the CSP, respectively (Fig. 31b). The modification of both structures of the profens and CSP alters the interaction of the chiral profen with the CSP (Fig. 31c). Moreover, with the acidic modifier, the higher order structure of the CSP is modified as well. The overall modification of the structures of profen and CSP by the acidic mobile phase modifier favors efficient interaction of profen with the CSP for chiral discrimination, thus, enhancing enantioselectivity.

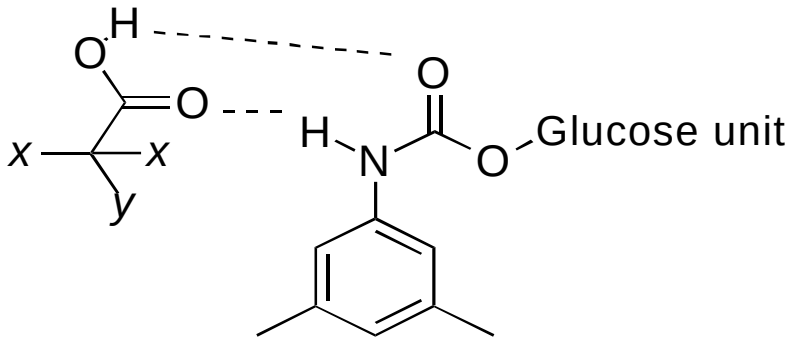
Modification of the structure of a racemic profen by an acidic modifier, such as TFA, may arise by association or solvation. Generally, carboxylic acids, such as profens, exist as dimers in both the liquid and vapor states[284] due to intermolecular hydrogen bonding between the carbonyl oxygen and hydroxyl groups of two acid molecules. The presence of the acidic modifier as the solvating species may prevent the association of a pair of profen enantiomers, thus enhancing the enantioseparation.

(a)



x = H or F; *y* = C_2F_5

(b)



x = H or F; *y* = C_2F_5

(c)

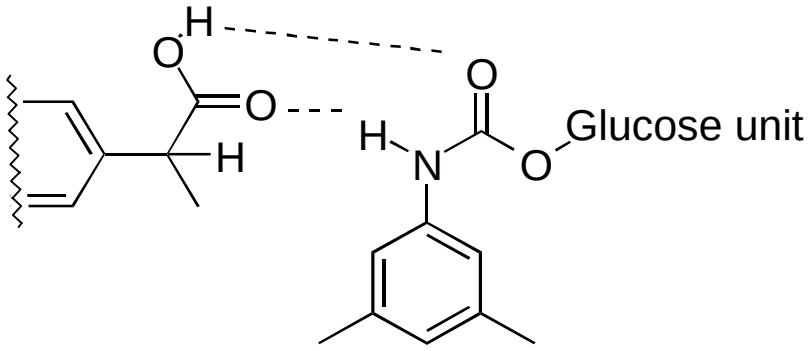


Figure 31. Hydrogen bonding interactions between (a) acidic mobile phase modifier and profen, (b) acidic mobile phase phase modifier and CSP, and (c) profen and CSP.

For the CSP, its interaction with the acid modifier by hydrogen bonding may change its higher order structure. In Chapter III, it is noted that the degree of swelling of derivatized polysaccharide CSPs depend on the dielectric constant and hydrogen bonding capability of the solvent. A carboxylic acid as a modifier form two hydrogen bonds with the CSP that may modify the size and geometry of the chiral cavities and consequently affects retention and enantioselectivity.

Without the acidic modifier, the profen and CSP are solvated by ethanol molecules. Ethanol can interact with the profen or CSP forming only one hydrogen bond for every ethanol molecule (Fig. 32). The modification of the structures of the analyte and CSP, including its higher order structure, by ethanol is different from that of any acidic mobile phase modifier.

Chiral separation of a racemate profen on Chiralpak AD involves the following: (1) attractive-repulsive interactions, such as (a) hydrogen bonding between the carbonyl oxygen and hydroxyl proton of profen carboxylic moiety and the amide proton and carbonyl oygen of the carbamate moiety of the CSP, respectively; (b) dipole-dipole interaction, and (c) π-π interaction between phenyl rings; and (2) steric fit of the molecule to the chiral cavity of the CSP. All of these interactions between a profen and CSP are present with and without an acidic mobile phase modifier. However, it is the modification of the structures of analyte and CSP, including the CSP higher order structure, that facilitates selective interaction for chiral discrimination.

The dramatic decrease of k_2 after the addition of acidic mobile phase modifiers strongly suggests that the modifiers compete with the analytes for the achiral and chiral active sites of the CSP, specifically for hydrogen bonding. Both racemic analytes and acidic mobile phase modifiers form two types of hydrogen bonding with the CSP, i.e., between the carbonyl oxygen and hydroxyl proton of the carboxyl group of the acid and the amide proton and carbonyl oxygen of the carbamate moiety of the CSP, respectively. However, the strength of hydrogen bond depends on the distance between the interacting molecules, which in turn is dependent on the following: (a) the proton donating ability of the atom or the electron withdrawing ability of the the group of atoms that hydrogen is

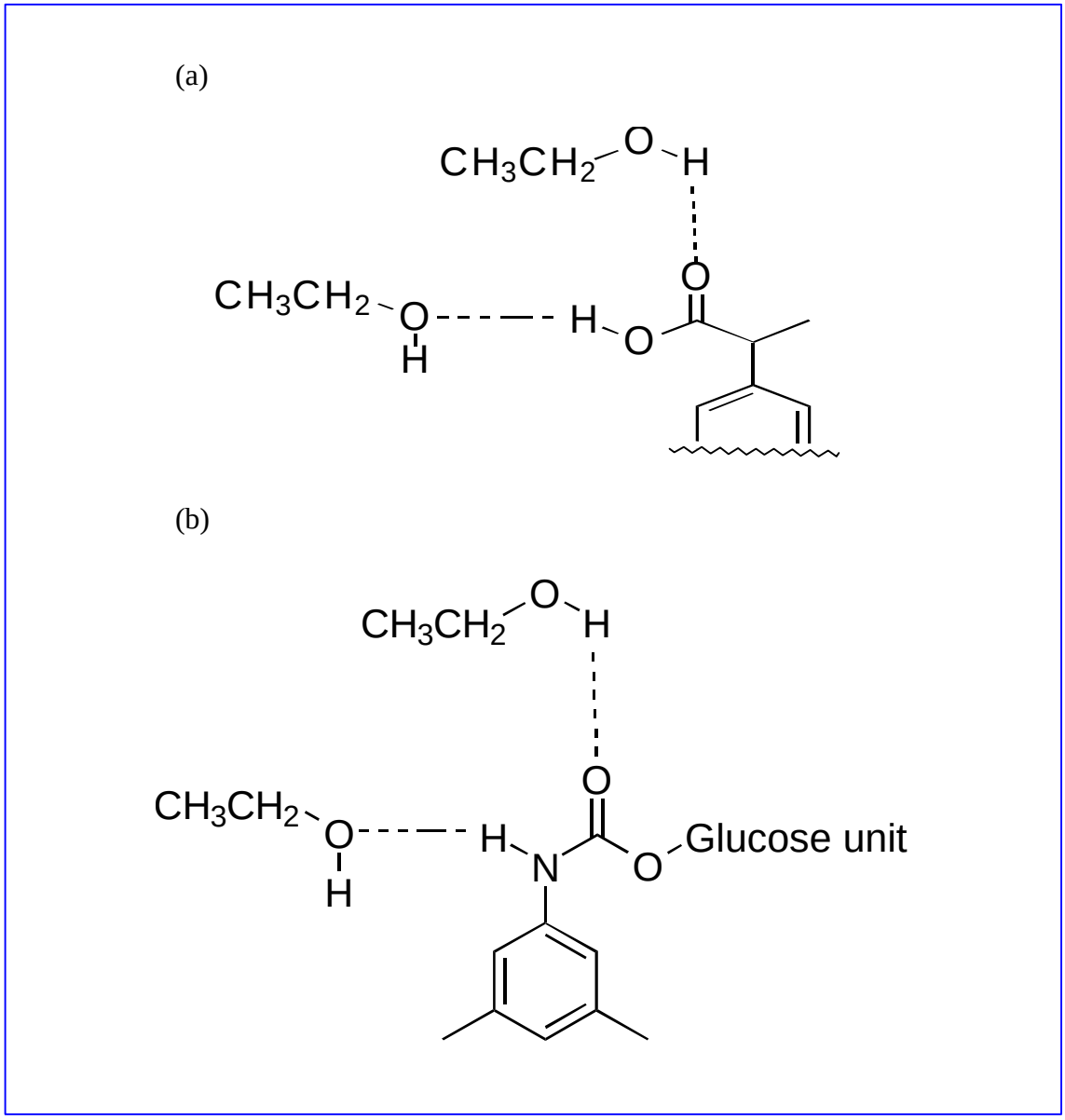


Figure 32. Possible hydrogen bonding interactions between (a) ethanol and profen and (b) ethanol and CSP.

bonded to; (b) the proton accepting ability of the receiving atom; and (c) molecular geometry. Among HOAc, HFBA, and TFA, HOAc forms the weakest hydrogen bonding because of the fact that it is the weakest acid. Hence the retention of the analytes are longer with HOAc than with HFBA or TFA as a mobile phase modifier. With either TFA or HFBA, the retention of analytes are the same probably because the acid modifiers have almost the same pKa (0.3 and 0.4, respectively). It appears that the size and the hydrophobicity of the alkyl chain of the acid modifiers do not affect retention, only their hydrogen bonding abilities that matter.

A decrease in retention is to be expected in normal phase chromatography when the hydrophobicity of analyte is increased, thus increasing the analyte's solubility in the mobile phase. In addition, reduction of analyte-CSP interactions will also decrease retention. In this case, replacement of the hydroxyl proton with a methyl group reduces the ability of the analyte to act as a hydrogen donor. The drastic decrease in k_2 when a profen is converted to its corresponding methyl ester (Table VIII) obviously points out the importance of the hydroxyl proton of the profen carboxylic moiety for retention. In addition, the hydroxyl proton of the carboxylic moiety plays a role in chiral discrimination. Except for the ketoprofen methyl ester, all the profen esters showed no enantioseparation while the corresponding racemic acids were enantioseparated at the same chroamtographic conditions. Nevertheless, the enantioseparation of the ketoprofen methyl ester with HFBA or TFA strongly indicates that the hydrogen between the profen keto oxygen and the amide proton of the carbamate moiety of the CSP (Fig. 33) is critical for chiral discrimination.

The enantioselectivity of profens on Chiralpak AD seems not to be influenced by the streric size of the acidic mobile phase modifier. There was no significant change of enantioselectivity of profens and profen methyl esters when TFA was changed to HFBA.. This may be due to the fact that the two acidic modifiers have comparable hydrogen bonding abilities, i.e., nearly the same pKa. The enantioselectivity of the analytes, therefore, is influenced by the hydrogen bonding abilities of the acidic modifiers. This conclusion is strongly supported by the fact that HOAC, a weaker acid than TFA or HFBA, as the modifier the enantioselectivity of profens is small. The improvement of

enantioselectivity by HFBA or TFA may be due to the modification of the structures of the analytes and the Chiralpak AD CSP that facilitates selective enantioseparation.

Although separation was not obtained for the methyl esters of fenoprofen and ibuprofen, this may be due the fact that the analyte-CSP interactions governing general retention were insufficient to permit noticeable discrimination between enantiomers. The k_2 of fenoprofen and ibuprofen methyl esters (Table VIII) are smaller as compared to the corresponding profens (Table VII) chromatographed at the same conditions. However, there was no significant change in the enantioselectivity and resolution of ketoprofen methyl ester with HFBA and TFA as acidic modifiers even when the analyte was more retained. This results strongly suggest that the hydrogen bond arising from the keto oxygen of ketoprofen is critical for chiral discrimination.

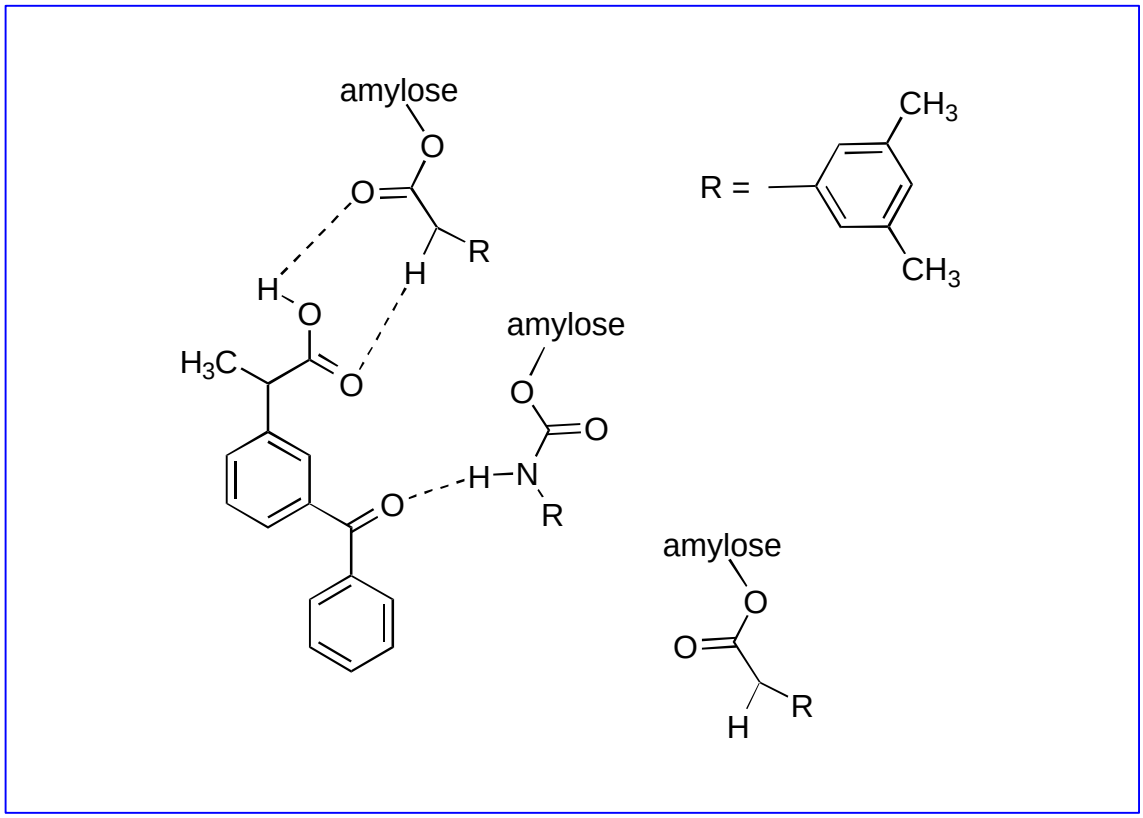


Figure 33. Proposed chiral recognition mechanism showing the hydrogen bonding of ketoprofen with Chiralpak AD.

As shown in Table IX, the enantioseparations of profens on Chiralcel OD following addition of HOAc, HFBA, and TFA are also characterized by a decrease in k_2 and an increase in α. Both changes in k_2 and α of profens with the presence of acidic modifier are significant averaging around 80 and 9 %, respectively. The change from HOAc to HFBA results to changes in k_2 and α that average about 38 and 3%, respectively. Changes in k_2 and α when HOAc is changed to TFA average around 33 and 3 %, respectively. There were no significant changes in either k_2 or α when HFBA was changed to TFA. As in Chiralpak AD, the size and hydrogen bonding ability of either HFBA or TFA appear to have no influence on k_2 and α. The overall changes average about 5 and 0.5%, respectively. In addition, the conversion of fenoprofen and ketoprofen to their corresponding methy ester results in a drastic decrease in retention that average about 197% (Table X). Lastly, all the profen methyl esters were not enantioseparated.

The resolution of racemic profens was facilitated upon addition of HOAc, HFBA, and TFA. The overall increase in resolution average around 59%. Changing HFBA to TFA as the acidic modifier yields insignificant change in resolution averaging about 5%.

The rationales for the marked decrease of retention and increase of enantioselectivity of the racemic profens separated on Chiralcel OD in the presence of acidic modifiers are the same as those for Chiralpak AD. The acidic modifier alters the structures of racemate profens and the CSP, including the CSP higher order structure, as well as competing with the analytes for active binding sites on the CSP. The reasons for the longer retention of profens with HOAc than with TFA of HFBA, and the drastic decrease in k_2 when the profens are converted to methyl esters on Chiralpak AD, hold true also for Chiralcel OD.

However, the mechanisms for the interaction of profens with Chiralcel OD and Chiralpak AD leading to enantiomeric separation may be different. As previously discussed, both CSPs consist of the same chiral adsorbing sites of tris(3,5-dimethylphenylcarbamate)-D-glucose residue but have different higher order structures. Chiralcel OD is regarded as a left handed three-fold helix arising from a β-(1,4) -D-glucose linkage and Chiralpak AD is a left handed four-fold helix (4/1) due to an α-(1,4)-D-glucose linkage[285, 286]. The enantiomers may interact with one or more 3,5-dimethyl-

Table IX. Influence of acidic mobile phase modifiers on retention and enantioselectivity of profens on Chiralcel OD.

Profen	Acidic Mobile Phase Modifier	k_2[a]	Enantio-selectivity α	Resolution **R**
Carprofen	None	3.38[b]	1.00[b]	0.00[b]
(1.72 M EtOH in	HOAc	$2.68 \pm 8.79 \times 10^{-3}$	$1.09 \pm 5.83 \times 10^{-3}$	$0.43 \pm 2.52 \times 10^{-2}$
hexane with 2.08×10^{-6}	HFBA	$1.79 \pm 8.94 \times 10^{-3}$	$1.16 \pm 8.31 \times 10^{-3}$	$0.86 \pm 4.31 \times 10^{-2}$
moles acid)	TFA	$1.88 \pm 1.08 \times 10^{-2}$	$1.15 \pm 1.06 \times 10^{-2}$	$0.84 \pm 5.00 \times 10^{-2}$
Fenoprofen	None	3.08[b]	1.00[b]	0.00[b]
(0.34 M EtOH in	HOAc	$2.82 \pm 8.58 \times 10^{-3}$	$1.11 \pm 4.84 \times 10^{-3}$	$0.89 \pm 4.18 \times 10^{-2}$
hexane with 1.95×10^{-6}	HFBA	$2.59 \pm 7.77 \times 10^{-3}$	$1.14 \pm 5.41 \times 10^{-3}$	$0.85 \pm 3.92 \times 10^{-2}$
moles acid)	TFA	$2.75 \pm 3.93 \times 10^{-3}$	$1.13 \pm 2.63 \times 10^{-3}$	$0.92 \pm 2.15 \times 10^{-2}$
Flurbiprofen	None	3.12	1.00	0.00
(0.34 M EtOH in	HOAc	$2.71 \pm 8.74 \times 10^{-3}$	$1.06 \pm 5.42 \times 10^{-3}$	$0.41 \pm 2.81 \times 10^{-2}$
hexane with 1.95×10^{-6}	HFBA	$2.01 \pm 1.19 \times 10^{-3}$	$1.09 \pm 7.52 \times 10^{-4}$	$0.65 \pm 3.88 \times 10^{-2}$
moles acid)	TFA	$2.25 \pm 2.56 \times 10^{-3}$	$1.08 \pm 1.70 \times 10^{-3}$	$0.61 \pm 1.09 \times 10^{-2}$
Ketoprofen	None[b]	11.90[b]	1.00	0.00
(0.34 M EtOH in	HOAc	$6.62 \pm 8.46 \times 10^{-3}$	$1.05 \pm 1.97 \times 10^{-3}$	$0.36 \pm 2.94 \times 10^{-2}$
hexane with 1.95×10^{-6}	HFBA	$4.35 \pm 3.49 \times 10^{-3}$	$1.08 \pm 1.02 \times 10^{-3}$	$0.65 \pm 1.00 \times 10^{-2}$
moles acid)	TFA	$4.36 \pm 5.26 \times 10^{-3}$	$1.08 \pm 1.90 \times 10^{-3}$	$0.64 \pm 1.00 \times 10^{-2}$

*Retention factor of the second eluted enantiomer.

Average of six runs 25°C, flow rate 1.0 mL/min. Exceptions are [b] which are average of two runs.

Table X. Influence of acidic mobile phase modifiers on retention and enantioselectivity of profen methyl esters on Chiralcel OD.

Profen	Acidic Mobile Phase Modifier	k_2*	Enantio-selectivity α	Resolution **R**
Fenoprofen Ester (0.34 M EtOH in hexane with 1.95 x 10^{-6} moles acid)	HFBA	0.71 ± 2.76 x 10^{-4}	1.00 ± 5.48 x 10^{-4}	0.00
	TFA	0.74 ± 5.03 x 10^{-4}	1.00 ± 9.64 x 10^{-4}	0.00
Ibuprofen Ester (0.34 M EtOH in hexane with 1.95 x 10^{-6} moles acid)	HFBA	0.09 ± 5.40 x 10^{-4}	1.00 ± 8.51 x 10^{-3}	0.00
	TFA	0.12 ± 4.15 x 10^{-4}	1.00 ± 4.27 x 10^{-3}	0.00
Ketoprofen Ester (0.34 M EtOH in hexane with 1.95 x 10^{-6} moles acid)	HFBA	1.25 ± 6.79 x 10^{-4}	1.00 ± 7.69 x 10^{-4}	0.00
	TFA	1.31 ± 5.49 x 10^{-3}	1.00 ± 5.95 x 10^{-3}	0.00

*Retention factor of the second eluted enantiomer.

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

phenyl carbamate moieties of one or more glucose residues of either CSP to facilitate separation.

One proof for different mechanisms in the chiral discrimination of profens on Chiralcel OD and Chiralpak AD is the different requirement of mobile phase composition of hexane/ethanol/acidic modifier needed to optimize enantioseparation (see Tables IV, VII, and IX). For example, racemic ketoprofen was well resolved on Chiralpak AD using 80/20/0.15 hexane/ethanol/TFA. On chiralcel OD, ketoprofen was best enantioseparated using 98/2/0.40 hexane/ethanol/ TFA.

Furthermore, the methyl esters of fenoprofen, ibuprofen, and ketoprofen analyzed on Chiralcel OD, even though the retention factors of profens are higher as compared to those on Chiralpak AD, were not enantioseparated. For example, there was no separation for the enantiomers of the ketoprofen ester. This may imply that the additional hydrogen bonding arising from the keto oxygen of ketoprofen, aside those from the carbonyl oxygen and hydroxyl proton of the carboxylic moiety, is not critical for chiral discrimination of these methyl esters.

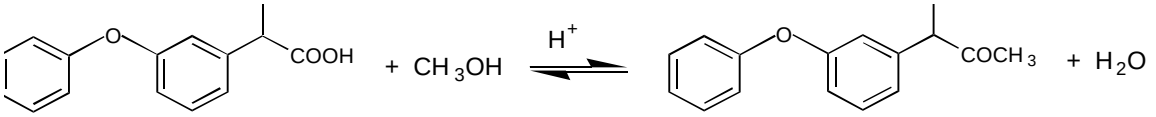
In summary, the addition of acidic mobile phase modifiers decreases the retention of profens on Chiralpak AD and Chiralcel OD but enhances their enantioselectivity. The role of the acidic mobile phase modifiers are the following: (1) they compete with the analytes for the achiral and chiral active sites of the CSP; and (2) they alter the structures of racemic profen and CSP, including the CSP higher order structure, thus promoting efficient interaction leading to chiral discrimination. TFA and HFBA, as acidic modifiers, gave the shortest retention but the greatest enantioselectivity and resolution of analytes. In both Chiralpak AD and Chiralcel OD, it appears that the dominant interaction of profens with the CSP for retention is the hydrogen bond arising from the acidic proton of its carboxylic moiety. This conclusion is strongly supported by the drastic decrease of retention when the profens are converted to methyl esters. On Chiralpak AD, the interaction that is critical for chiral recognition may be the additional hydrogen bond that does not arise from the carboxyl moiety of the profen. A proof for this is the enantioresolution of the methyl ester of ketoprofen. On the other hand, on Chiralcel OD,

the additional hydrogen bond may not be important for chiral recognition. The racemic fenoprofen, ibuprofen, and ketoprofen methyl esters were not separated.

5.31a Synthesis and Identification of Profen methyl Esters

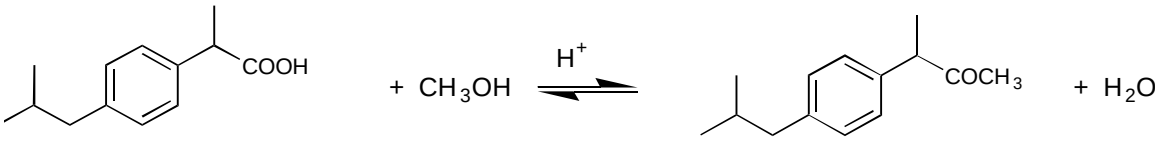
The methyl esters of fenoprofen, ibuprofen, and ketoprofen were syntheisized since they are not commercially available. The Fischer esterification reactions are the following:

(1)



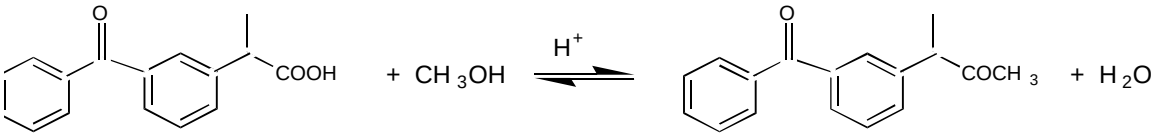
Fenoprofen Methanol Fenoprofen Methyl Ester

(2)



Ibuprofen Methanol Ibuprofen Methyl Ester

(3)



Ketoprofen Methanol Ketoprofen Methyl Ester

Verification of the purity and identity of the synthesized profen methyl esters was through GC-MS. The total ion chromatograms and mass spectra of the profen methyl esters are shown in Fig. 34, 35, and 36.

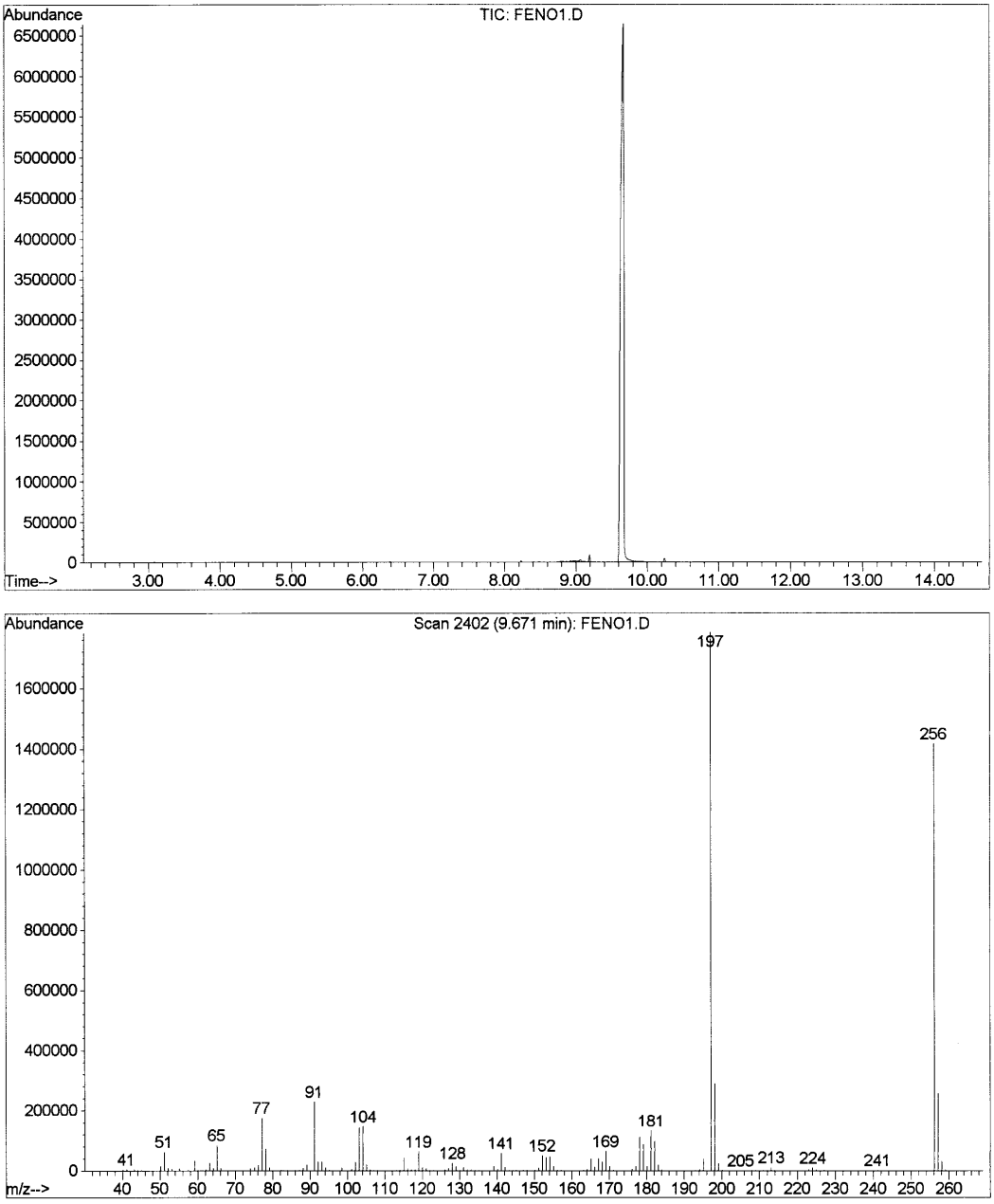


Figure 34. Total ion chromatogram and mass spectrum of the synthesized fenoprofen methyl ester.

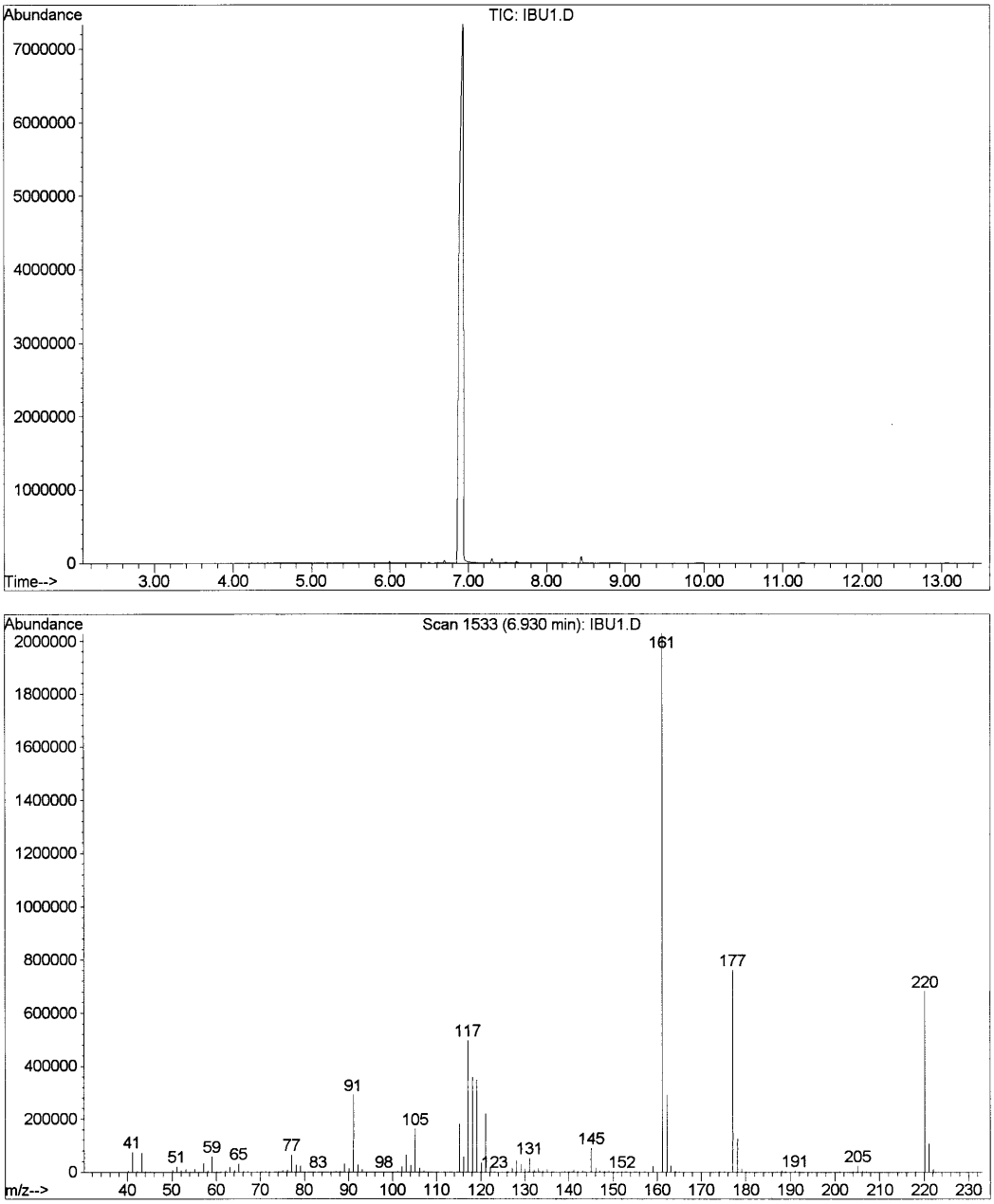


Figure 35. Total ion chromatogram and mass spectrum of the synthesized ibuprofen methyl ester.

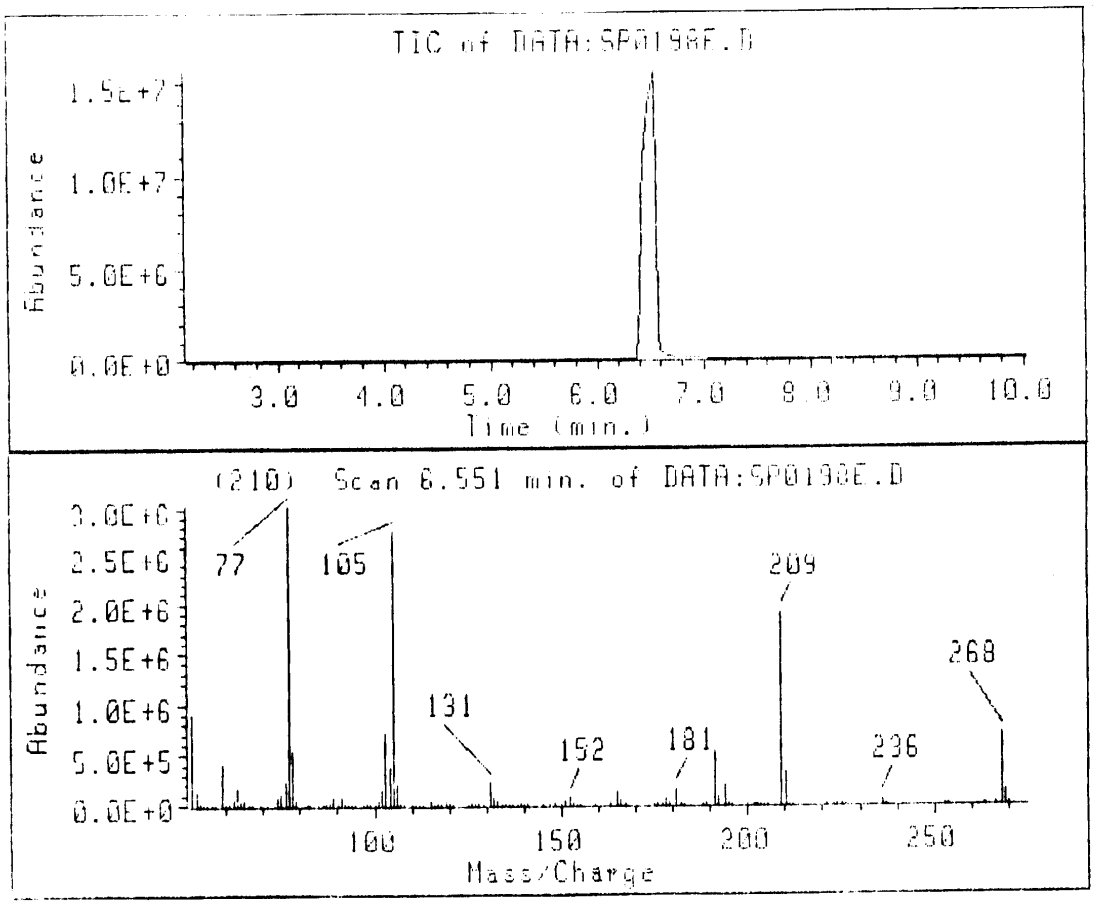


Figure 36. Total ion chromatogram and mass spectrum of the synthsized ketoprofen methyl ester.