# CHAPTER IV

RESULTS AND DISCUSSION

4.1 Synthesis of phenoxy acid methyl ester derivatives

Phenoxy acid methyl esters studied in this research were synthesized from the reaction between phenols and methyl 2-bromopropionate in the presence of potassium carbonate and acetone. Phenoxy acid methyl esters were produced via nucleophilic substitution reaction. Carbonate ions in the solution help to generate phenoxide ions that act as nucleophile attacking the partially positive carbon atom connected to the electronegative bromo group ($\mu_{aliphatic\ C\text{-}Br}$ = 1.82 D) [44]. The products were purified by column chromatography, if necessary. Most synthesized products were acquired in approximately 50-70 % yield, with a few exceptions. Generally, mono-substituted phenols containing strong electron withdrawing group tended to react completely with methyl 2-bromopropanoate and provided higher yield than others.

The identity of all synthesized products was confirmed by ^{1}H-NMR using chemical shift values of three different protons in their molecules: **H^a**: CHC$\underline{H}_3$ at δ ~1.60-1.80 ppm (3H, d), **H^b**: OC$\underline{H}_3$ at δ ~3.70-3.90 ppm (3H, s) and **H^c**: C$\underline{H}$CH$_3$ at δ ~4.5-4.9 ppm (1H, q). Other aromatic protons appeared at 6.10-8.60 ppm with different patterns depending on the number and position of the substituent on an aromatic ring.
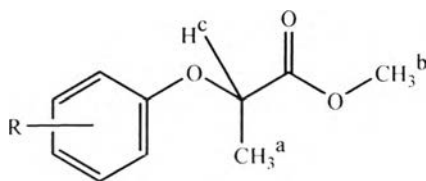
4.2 Determination of coated capillary column performance

The performance of capillary columns prepared for this research was evaluated by means of Grob test [41-42]. Test chromatograms obtained from OV-1701, BSiMe and BSiAc columns are displayed in figures 4.1- 4.3, respectively. The grob test mixture consists of twelve compounds with different functional groups: n-decane (C_{10}); n-undecane (C_{11}); methyl decanoate (E_{10}); methyl undecanoate (E_{11}); methyl dodecanoate (E_{12}); nonanal (al); 1-octanol (ol); 2,3-butanediol (D); 2,6-dimethylphenol (P); 2,6-dimethylaniline (A); 2-ethylhexanoic acid (S); and dicyclohexylamine (am). From GC chromatogram, column efficiency was determined from the average separation number (SN) values of ester peaks (E_{10}-E_{12}). The column inertness was evaluated from peak adsorption of alcohols (ol and D) and aldehyde (al). Acid-base property of column was examined through peak height ratio as well as peak adsorption of acid and base compounds (A, P, S and am).

According to figure 4.1, OV-1701 column shows high efficiency with an average SN value of 28.3 and the column efficiency determined isothermally at 80 and 200°C with n-alkanes is very good as well (3,000-4,000 plates/meter, $k' \geq 4$). These values confirm that OV-1701 column can be used efficiently at both low and high temperatures. The Grob test chromatogram depicts that this column is appropriate for quantitative analysis of monoalcohols (no adsorption observed). However, diol (D) and aldehyde (al) are slightly adsorbed, indicating separations of alcohols other than monoalcohols and of aldehydes with this column may not be suitable. Considering the peak height of P and A, they are rather equivalent revealing the column neutrality. Nonetheless, this column is very active to strong acid (S) and strong base (am) as seen from peak tailing; therefore, underivatized carboxylic acids and amines should not be directly analyzed on this stationary phase.
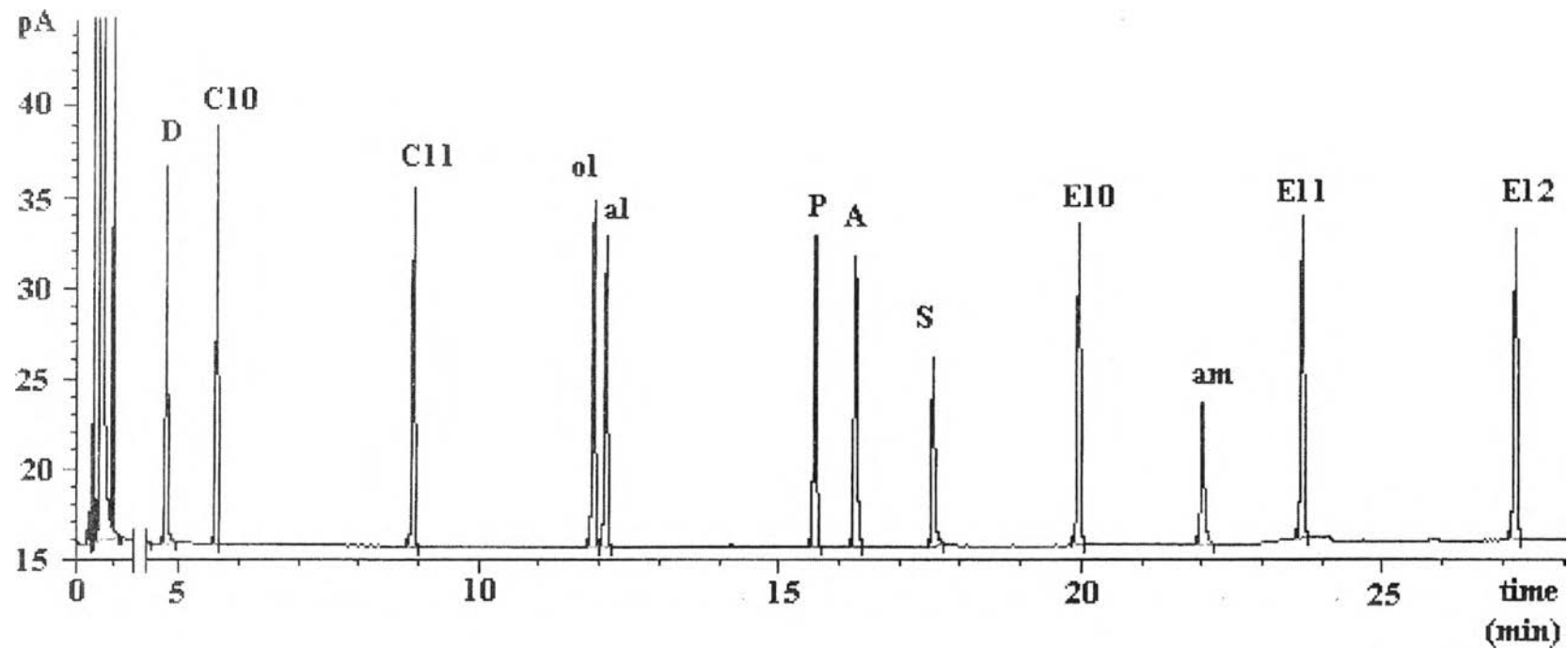
Figure 4.1   Chromatogram of Grob test on OV-1701 column (15.70 m × 0.25mm i.d. × 0.25 μm film thickness); temperature program: 40 to 150 °C at 3.18 °C/min.
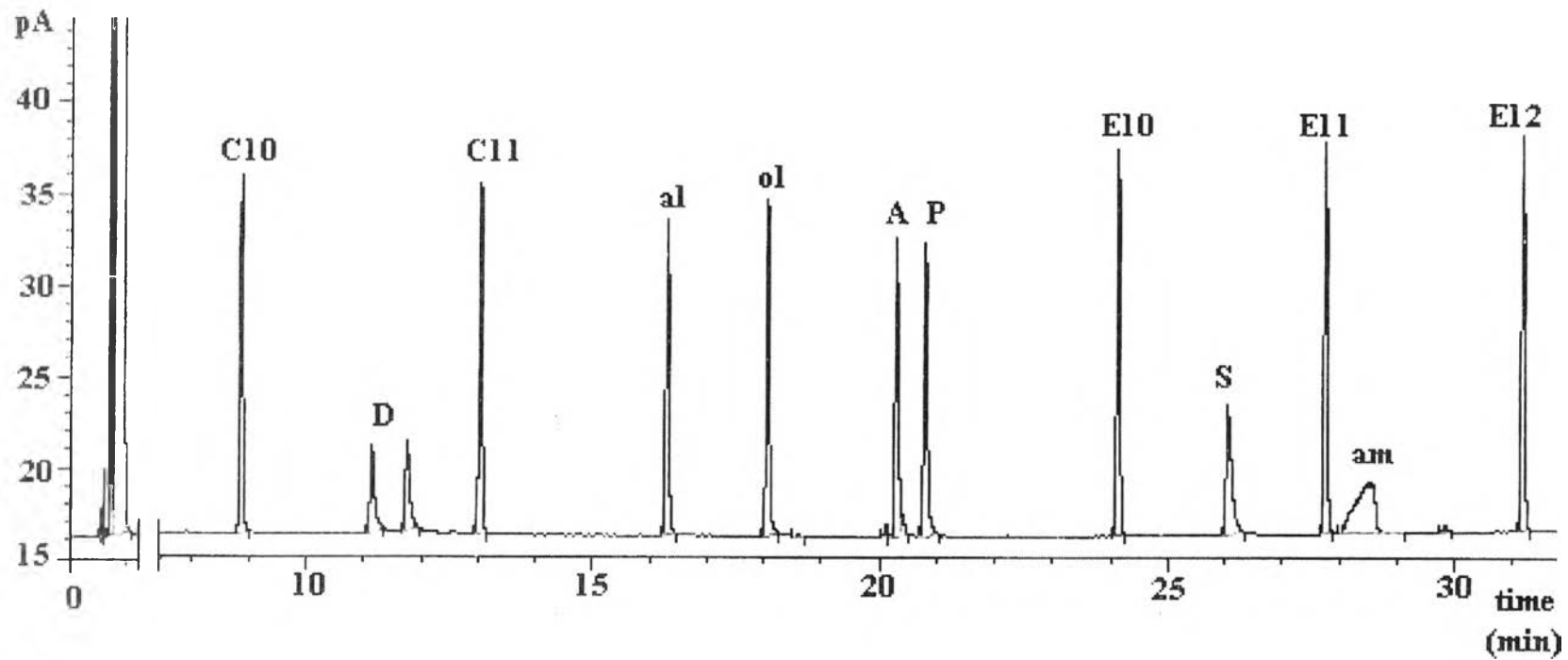
Figure 4.2 Chromatogram of Grob test on BSiMe column (15.75 m × 0.25 mm i.d. × 0.25 μm film thickness); temperature program: 40 to 150 °C at 3.16 °C/min.
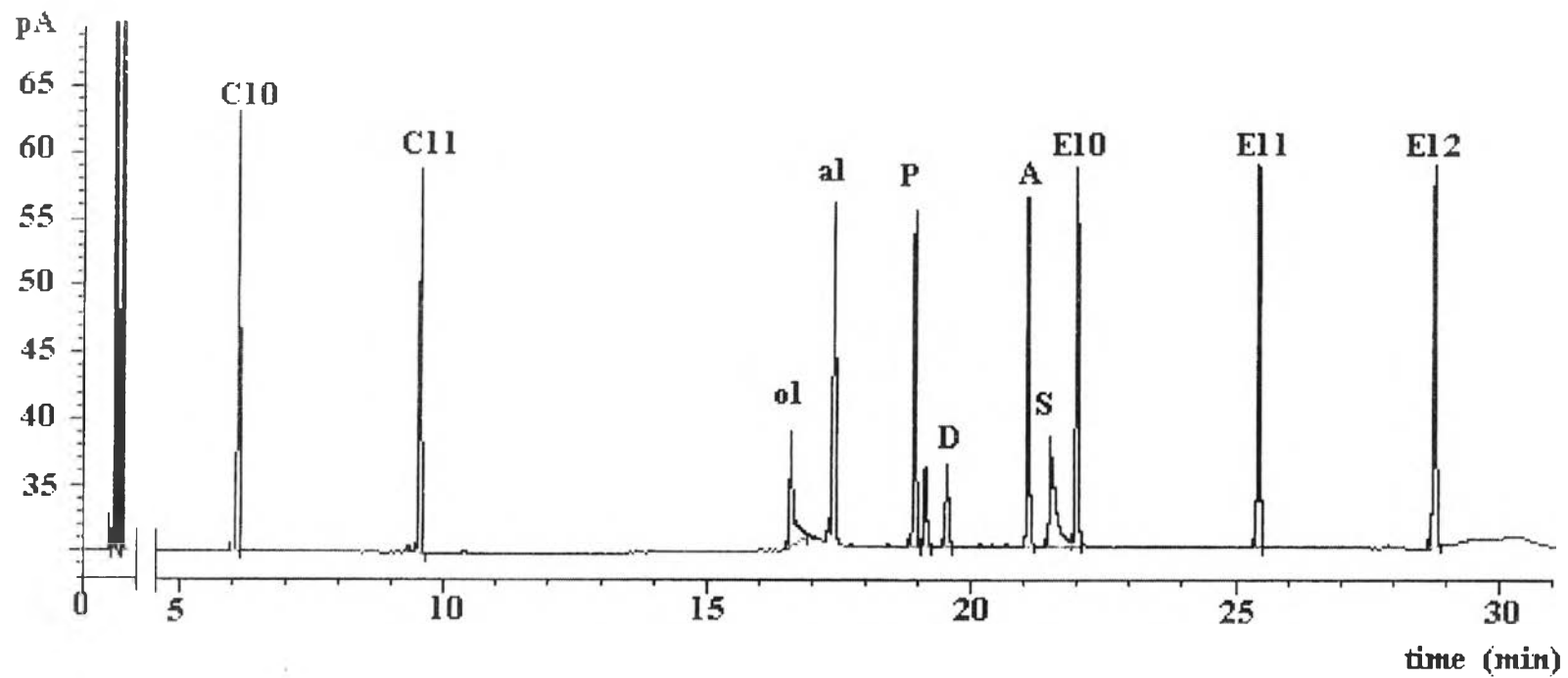
Figure 4.3 Chromatogram of Grob test on BSiAc column (16.00 m × 0.25 mm i.d. × 0.25 μm film thickness); temperature program: 40 to 150 °C at 3.12 °C/min.

As shown in figure 4.2, the efficiency of BSiMe column is rather high over a wide range of temperature with an average SN value of 28.5. The column neutrality is good as demonstrated by the similar peak height of **A** and **P**. Slight adsorption of aldehyde (**al**) and alcohols (**ol, D**) was observed, but the strong adsorption of **S** and **am** emerges on this stationary phase denoting that BSiMe column is not appropriate for separation of underivatized carboxylic acids and amines. Interestingly, the chiral compounds (**D**) displayed two peaks of enantiomers disclosing ability of derivatized cyclodextrin to separate enantiomeric compounds. Furthermore, the elution order of the test mixture attaining from this column differed from that on OV-1701 column.

As illustrated in figure 4.3, BSiAc column also shows high efficiency with an average SN value of 28.7 and the column neutrality is still good. However, this column is highly active towards alcohols (**ol, D**), strong acid (**S**) and strong base (**am**), all peaks of which are poorly eluted or vanished. Thus, this column is not suitable for the separation of underivatized alcohols, acids and amines. Nonetheless, BSiAc column can be employed for the analysis of esters, compounds of interest in this research, and it offers different selectivity to analytes since the elution order of tested compounds on this phase differs from that on BSiMe column.

These three columns could be performed efficiently with the reasonable analysis time for the separation of phenoxy acid methyl esters focused in this research.

4.3 Gas chromatographic separation of phenoxy acid methyl ester derivatives

All separations on three columns were performed isothermally, at least in duplicate, at 10 °C intervals in the temperature range of 90-220 °C. Considering the chromatographic results at the same operating temperature, the retention factors (k') and separation factors (α) of phenoxy acid methyl ester racemates on three columns (OV-1701, BSiMe and BSiAc) were compared at 170 °C and presented in figures 4.4-4.8.

The retention factors (k') of all analytes on each column varied significantly depending on the aromatic substituents. On the whole, phenoxy acid methyl esters with bromo, nitro and cyano substituents were highly retained in all columns; whereas fluoro, methyl and trifluoromethyl phenoxy acid methyl esters possessed smaller retention values. For the mono-substituted analytes with halogen substituent (fluoro, chloro, bromo), the retention factors (k') were considerably increased in order of fluoro < chloro < bromo following the size and molecular weight of substituent. The retention factors (k') of chloro-substituted analytes gradually increased in order of monochloro < dichloro < trichloro with the increasing number of chlorine atom on an aromatic ring. As shown in figures 4.4-4.6, the retention factors (k') of most mono-substituted analytes were commonly increased in the order of ortho- < meta- < para- isomers demonstrating that substituent position also impacted on analyte retentions. Additionally, it was apparently noticed that most analytes show higher retention values on two chiral columns than on the polysiloxane OV-1701 column, whereas all columns employed polysiloxane as a major component in the stationary phases with identical film thickness. Therefore, the additional interactions would be derived from cyclodextrin derivatives diluted in the stationary phase.
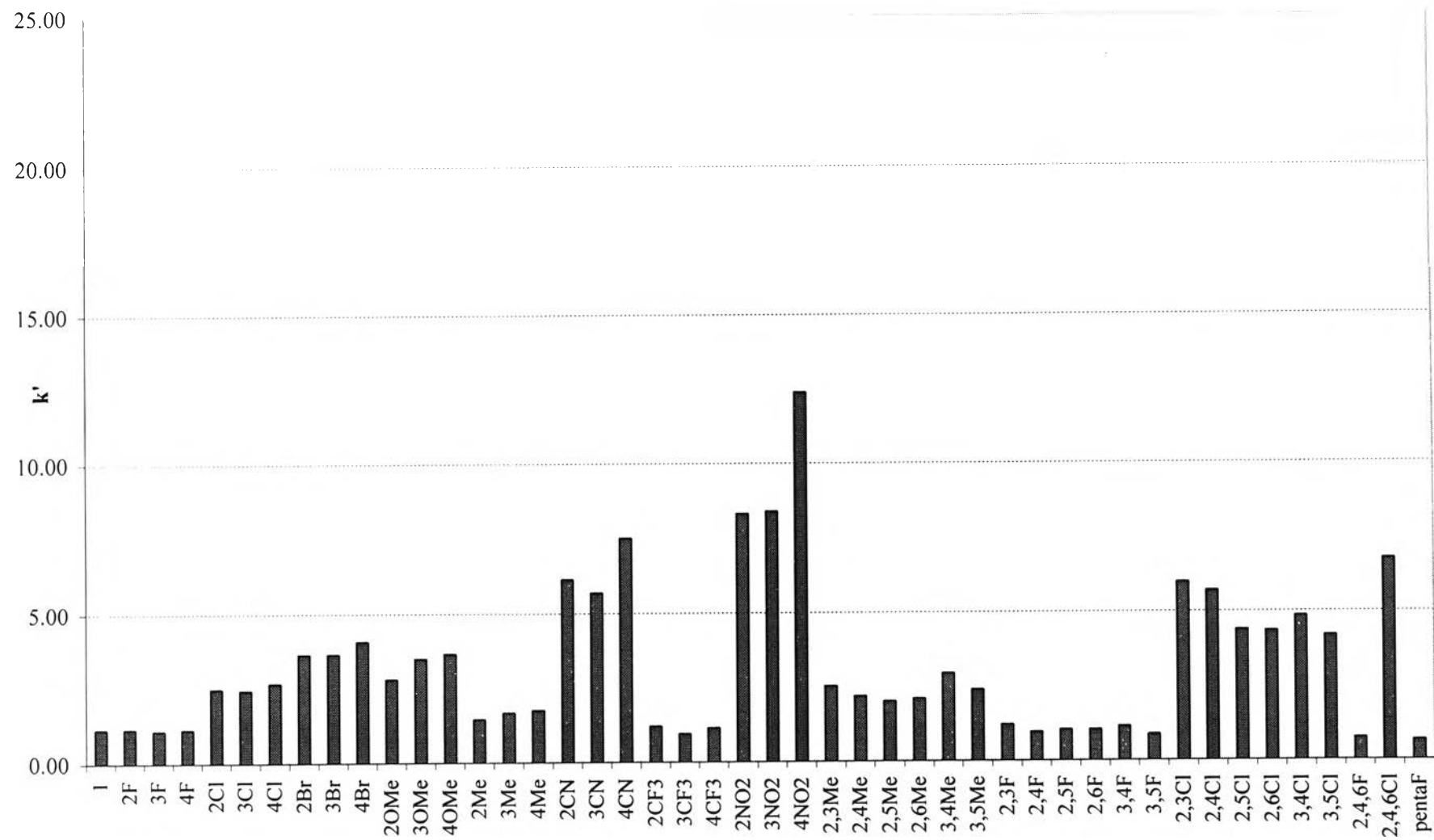
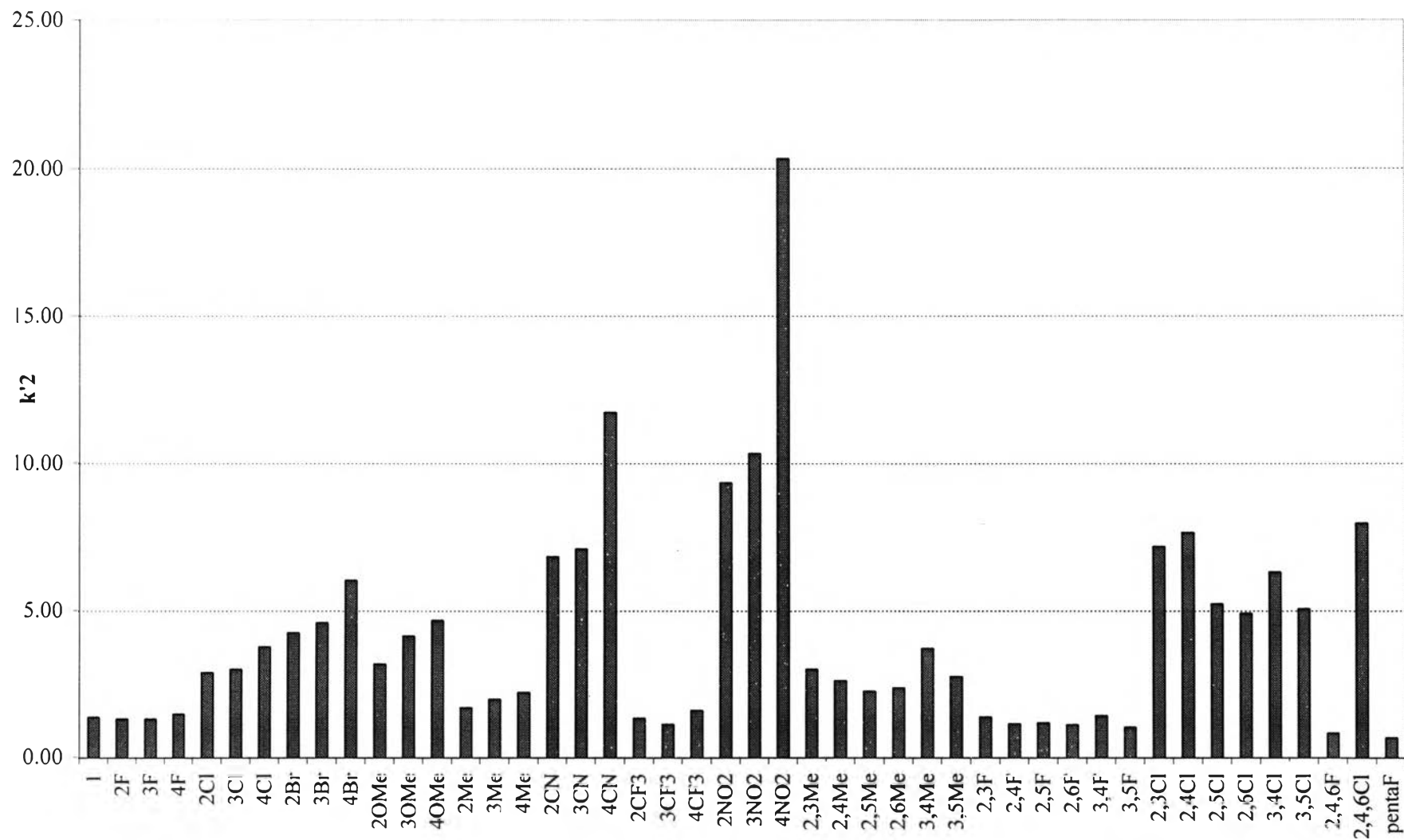**Figure 4.4**   Retention factors (k′) of phenoxy acid methyl esters on OV-1701 column at 170°C.

Figure 4.5   Retention factors (k′) of the more retained enantiomers of phenoxy acid methyl esters on BSiMe column at 170°C.
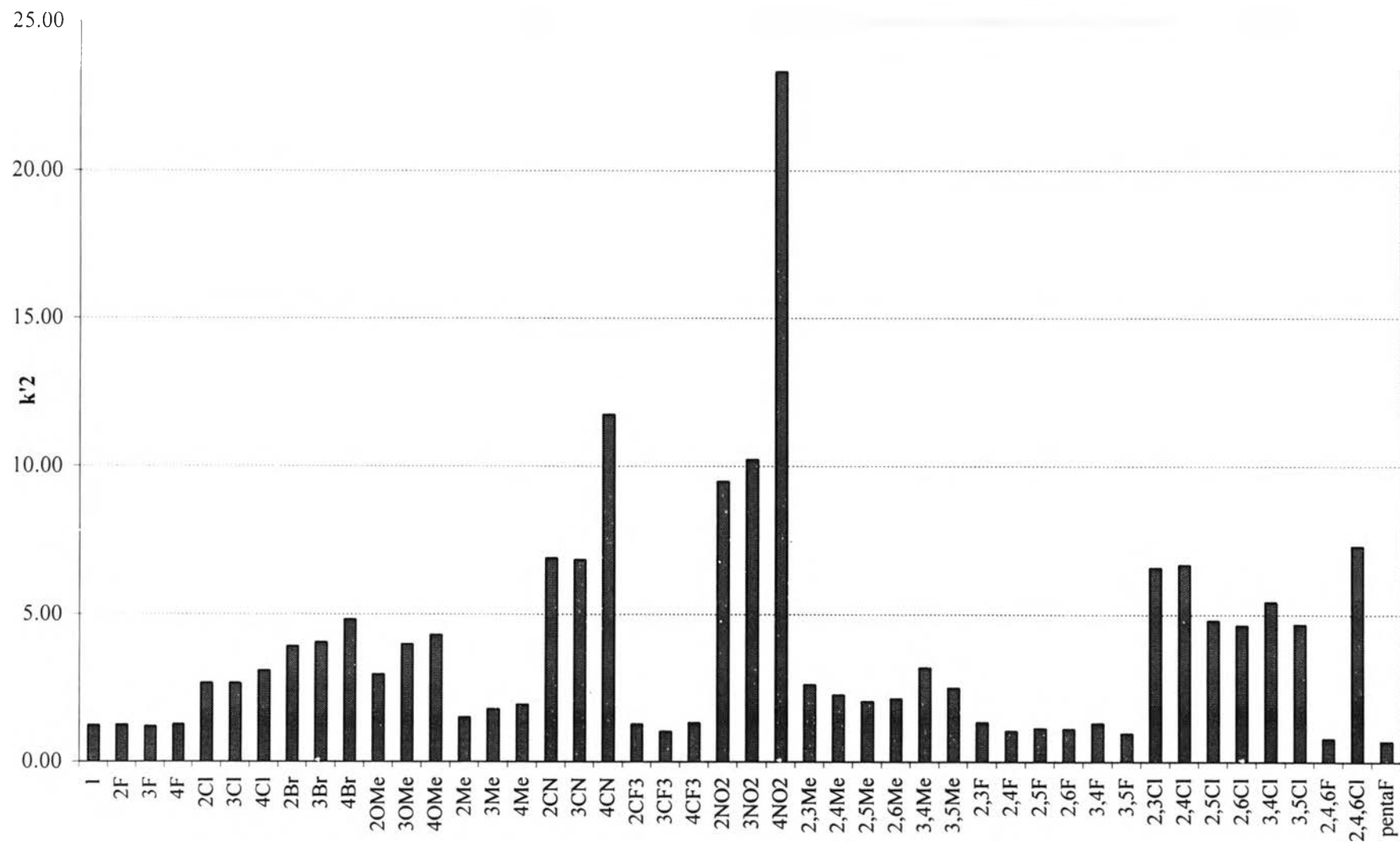
Figure 4.6 Retention factors (k′) of the more retained enantiomers of phenoxy acid methyl esters on BSiAc column at 170°C.
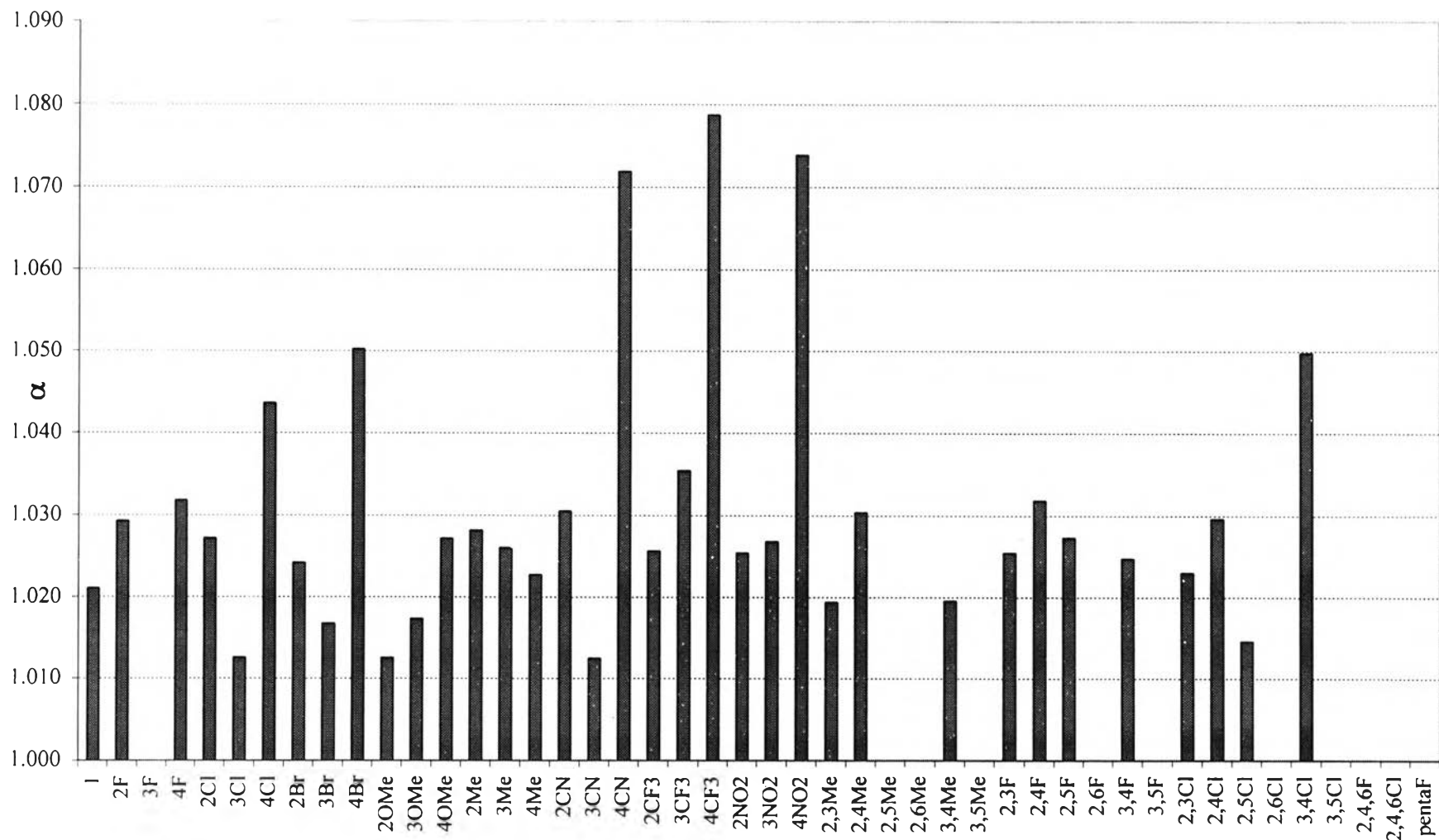
Figure 4.7 Separation factors (α) of the enantiomeric pairs of phenoxy acid methyl esters on BSiMe column at 170°C.
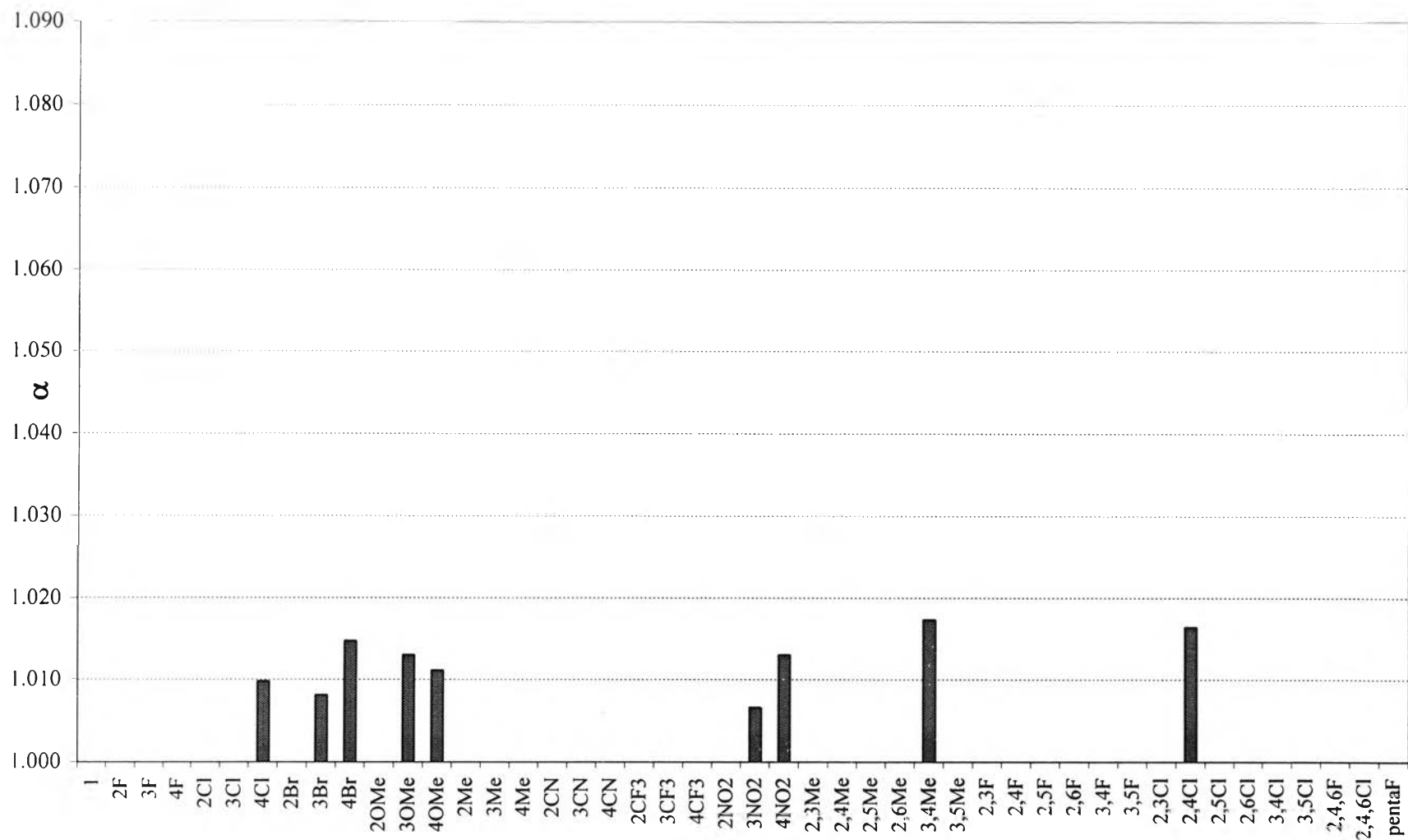
Figure 4.8 Separation factors (α) of the enantiomeric pairs of phenoxy acid methyl esters on BSiAc column at 170°C.

Considering the separation factors (α) at 170 °C (figures 4.7-4.8), both chiral columns (BSiMe and BSiAc) offered different enantioselectivity for each analyte. BSiMe exhibited fairly high separation factors (α) for most phenoxy acid methyl ester derivatives. Besides, all resolved analytes on BSiAc could be separated by BSiMe with a greater degree of separation. However, in some cases, BSiAc offered the superior results for analytes that are poorly separated by BSiMe phase, such as **3,5F**. The GC chromatograms of **3,5F** on both chiral columns at 110°C are illustrated in figure 4.9. It is clearly demonstrated that **3,5F** enantiomers could be perfectly separated by BSiAc column in shorter analysis time and with higher enantioselectivity than BSiMe column.
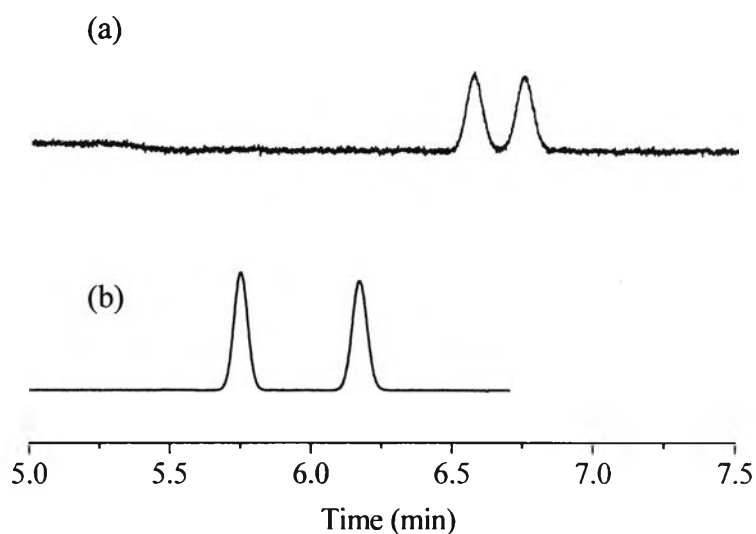
Figure 4.9   Chromatograms of **3,5F** on (a) BSiMe and (b) BSiAc columns at 110°C.

It can be seen that, the physical properties of the analytes, such as boiling point and vapor pressure, are substantially different, information from retention factors and enantioselectivities at specific temperature were not sufficient to reveal the nature of all interactions between analytes and cyclodextrin derivatives. Thus, thermodynamic parameters over a temperature range should be investigated to obtain additional details about the interactions between the analytes and gas chromatographic stationary phases [43].

4.4   Thermodynamic investigation by van't Hoff approach

To inspect the influences of the analyte structure on interaction strength and enantioresolution, thermodynamic parameters responsible for the analyte-stationary phase interactions and enantioresolution were achieved through van't Hoff plot of ln k' versus $1/T$, assuming an invariant heat capacity over the range of operating temperatures. All relationships between ln k' versus $1/T$ were linear with the correlation coefficient (R^2) greater than 0.990. The enthalpy (ΔH) and entropy (ΔS) values for each enantiomer could be calculated from the slope and y-intercept, respectively. When the enantiomeric pairs were separated, the corresponding $\Delta(\Delta H)$ and $\Delta(\Delta S)$ values could be determined from the relationship between ln α and $1/T$. Theoretically, these plots should be linear; however, the curvatures were observed in the temperature range examined for many analytes and caused errors in calculated thermodynamic values. Alternatively, the $\Delta(\Delta H)$ and $\Delta(\Delta S)$ values could be calculated from the difference in ΔH and ΔS values of two enantiomers derived from van't Hoff plot; therefore, the $\Delta(\Delta H)$ and $\Delta(\Delta S)$ values in this research were acquired through the latter approach instead. Nevertheless, these values obtained by both approaches were relatively similar.

4.4.1 Enthalpy change values (-ΔH) and entropy change values (-ΔS)

The enthalpy value (-ΔH) indicates the strength of interaction between an analyte and a stationary phase: the larger the value (more negative value), the stronger the interaction. While the entropy value (-ΔS) signifies the loss of degree of freedom associated with the interaction between an analyte and a stationary phase.

Enthalpy and entropy values of analytes obtained from OV-1701 column are illustrated in figures 4.10-4.11. It was observed that all analytes had very similar enthalpy values (-ΔH) within 13.60 ± 0.83 kcal/mol. This suggested that they all interacted with the OV-1701 reference stationary phase in a similar manner and the major contribution of analytes towards the interactions on this phase would probably come from ester and phenyl groups. It was observed that analytes possessing strong electron withdrawing group i.e. nitro and cyano (**2NO₂, 3NO₂, 4NO₂, 2CN, 3CN, 4CN**) exhibited higher enthalpy values than others. The interaction strength of positional isomers were generally in order of *meta- ≈ para- > ortho-* isomers and similar results were acquired with entropy values (-ΔS) as well.

Enthalpy (-ΔH) and entropy (-ΔS) values attained from two chiral columns (figures 4.12-4.15) were higher than those obtained from OV-1701 stationary phase. The higher -ΔH values on chiral columns indicated the enhancement of interaction between analytes and cyclodextrin derivatives. Nevertheless, resembling trends as in OV-1701 column were still recognized. It is interesting to note that *para-*isomers of mono-substituted analytes gave highest enthalpy and entropy values suggesting that *para*-substitution may cause an appropriate conformation of analyte to form a more stable inclusion complex intermediate with BSiMe and BSiAc phases.
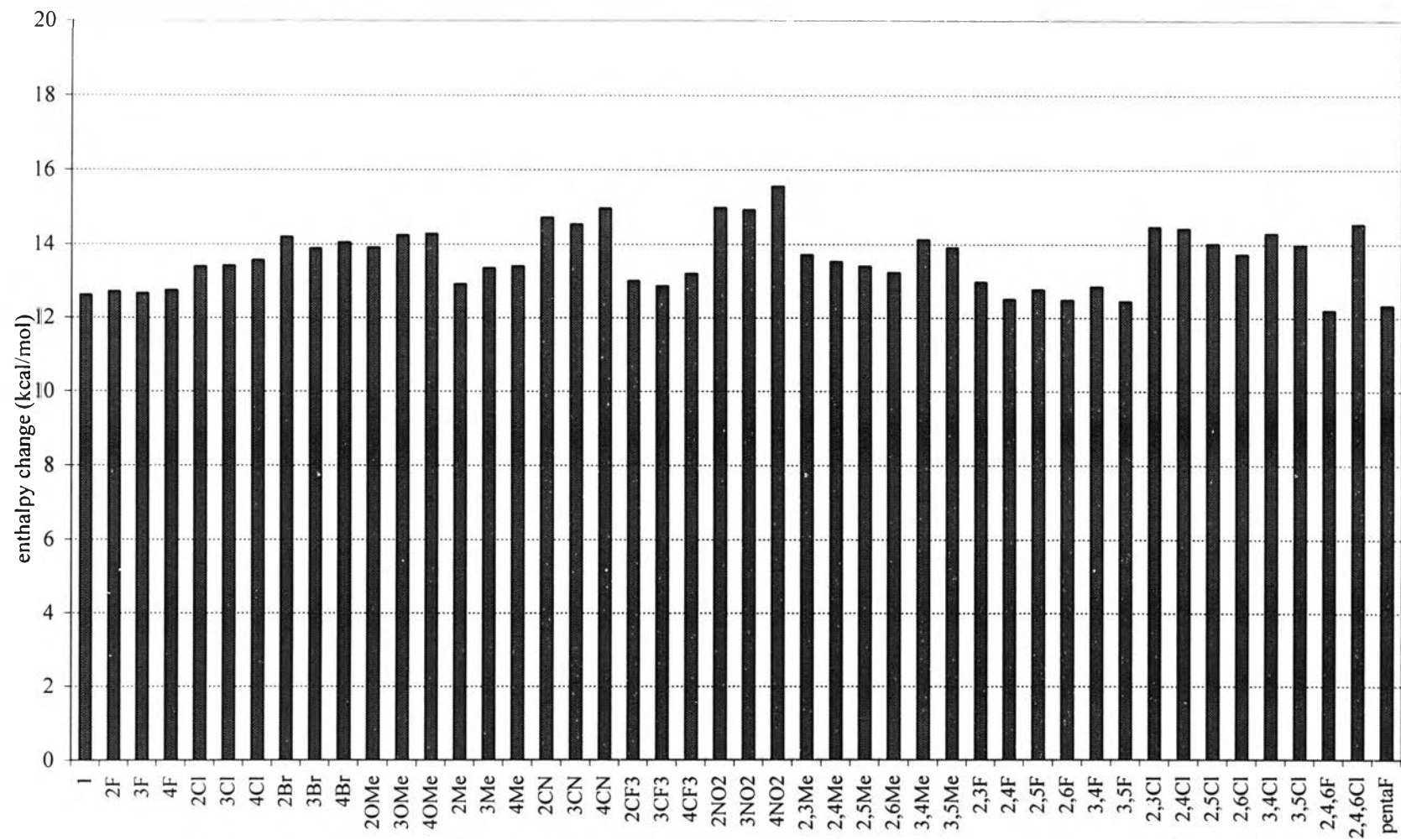
Figure 4.10 Enthalpy change ($-\Delta H$, kcal/mol) of phenoxy acid methyl esters on OV-1701 column obtained from van't Hoff approach ($\bar{x} = 13.60$; SD = 0.83).
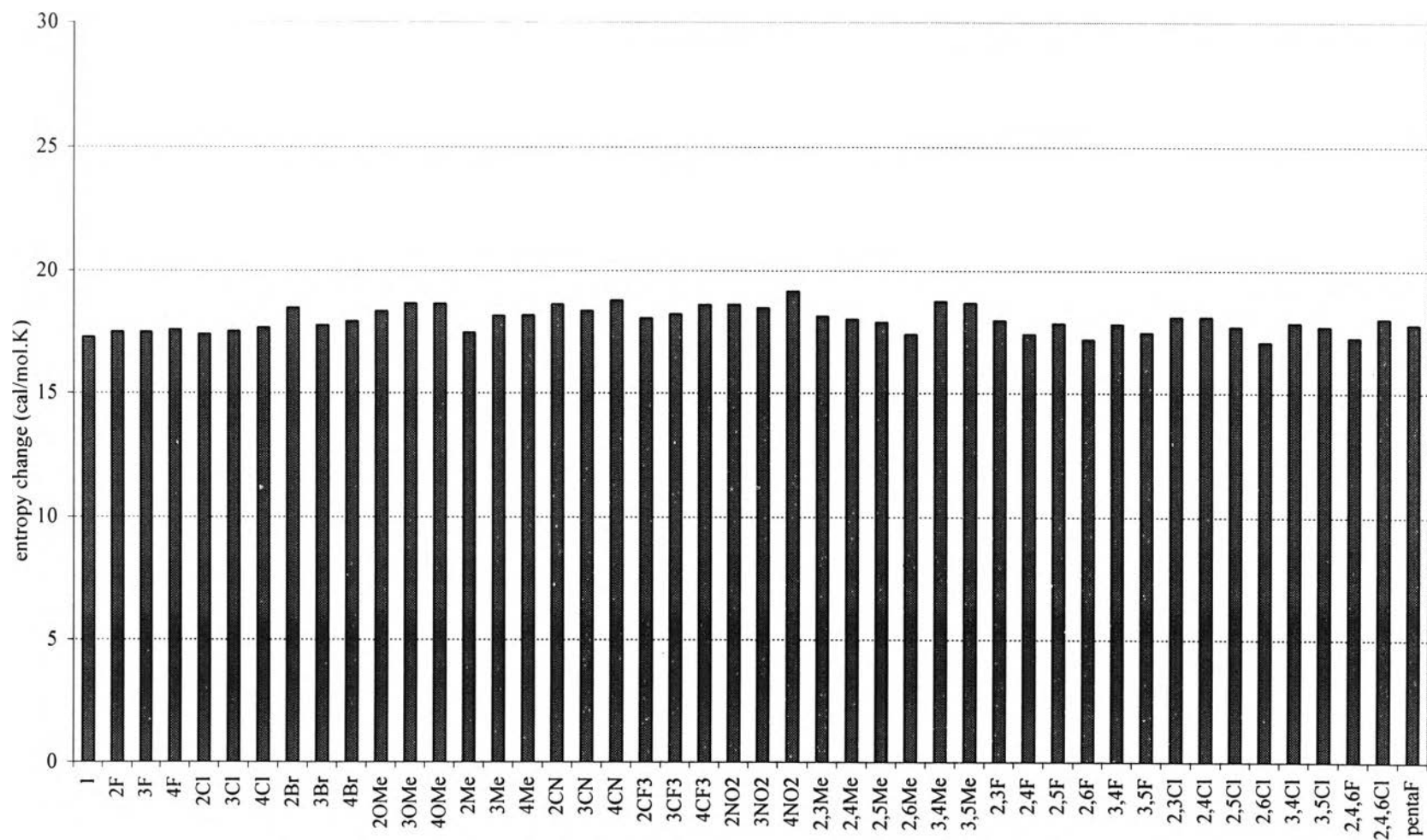
Figure 4.11 Entropy change (-ΔS, cal/mol·K) of phenoxy acid methyl esters on OV-1701 column obtained from van't Hoff approach ($\bar{x}$ = 17.99; SD = 0.50).
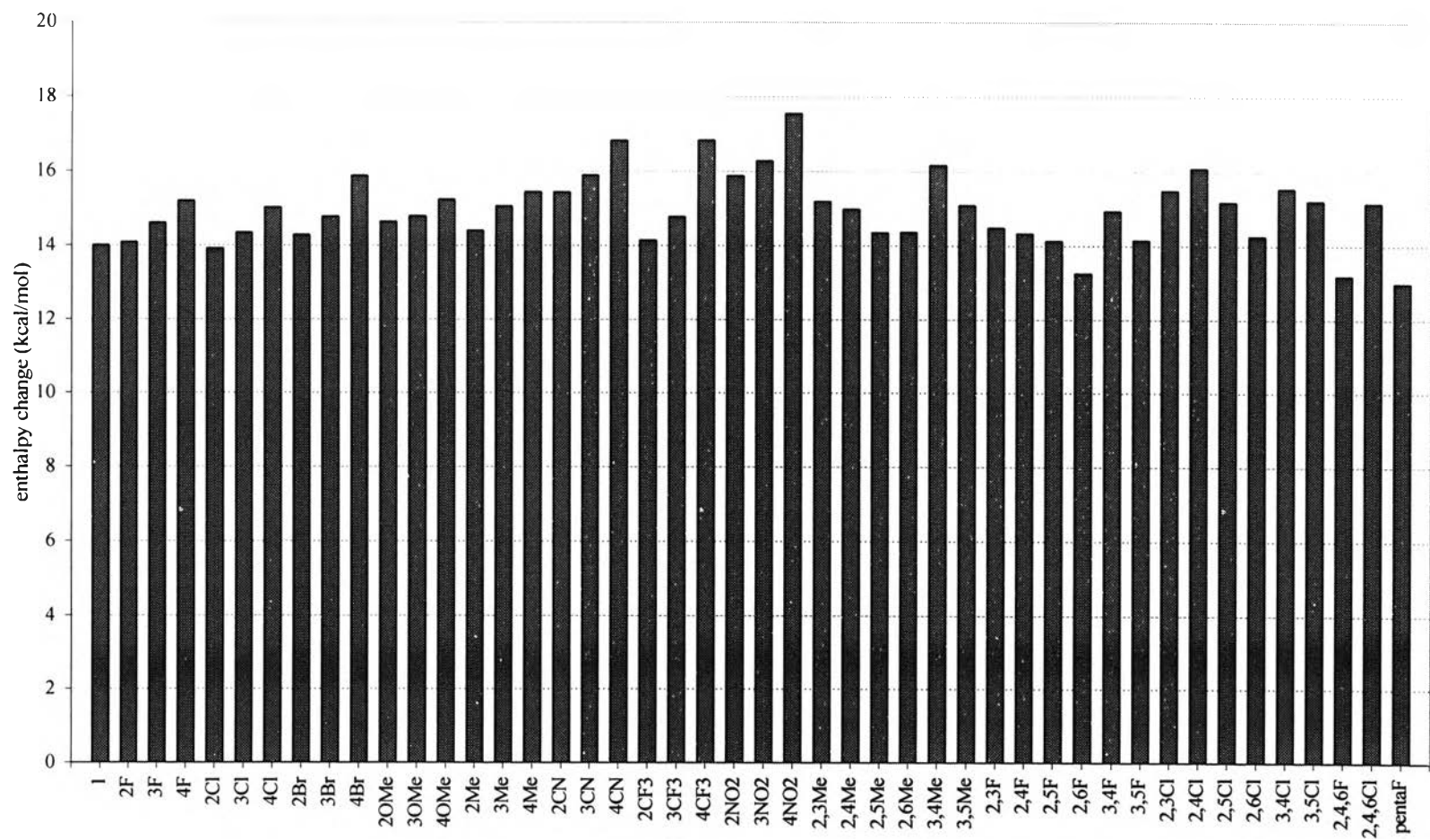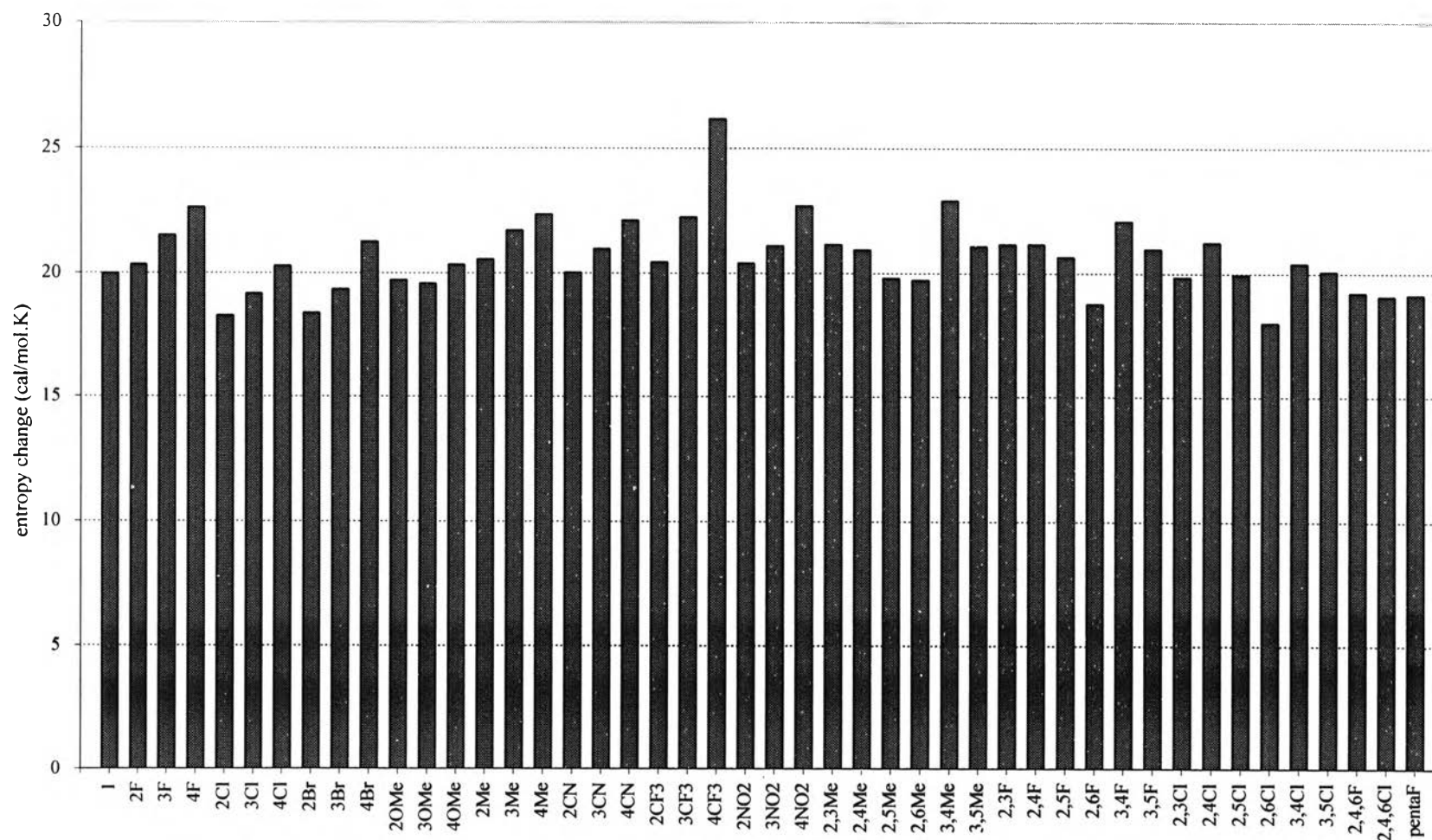
Figure 4.12 Enthalpy change ($-\Delta H_2$, kcal/mol) of the more retained enantiomers of phenoxy acid methyl esters on BSiMe column obtained from van't Hoff approach ($\bar{x}$ = 14.94; SD = 0.95).

Figure 4.13 Entropy change ($-\Delta S_2$, cal/mol·K) of the more retained enantiomers of phenoxy acid methyl esters on BSiMe column obtained from van't Hoff approach ($\bar{x} = 20.61$; SD = 1.46).
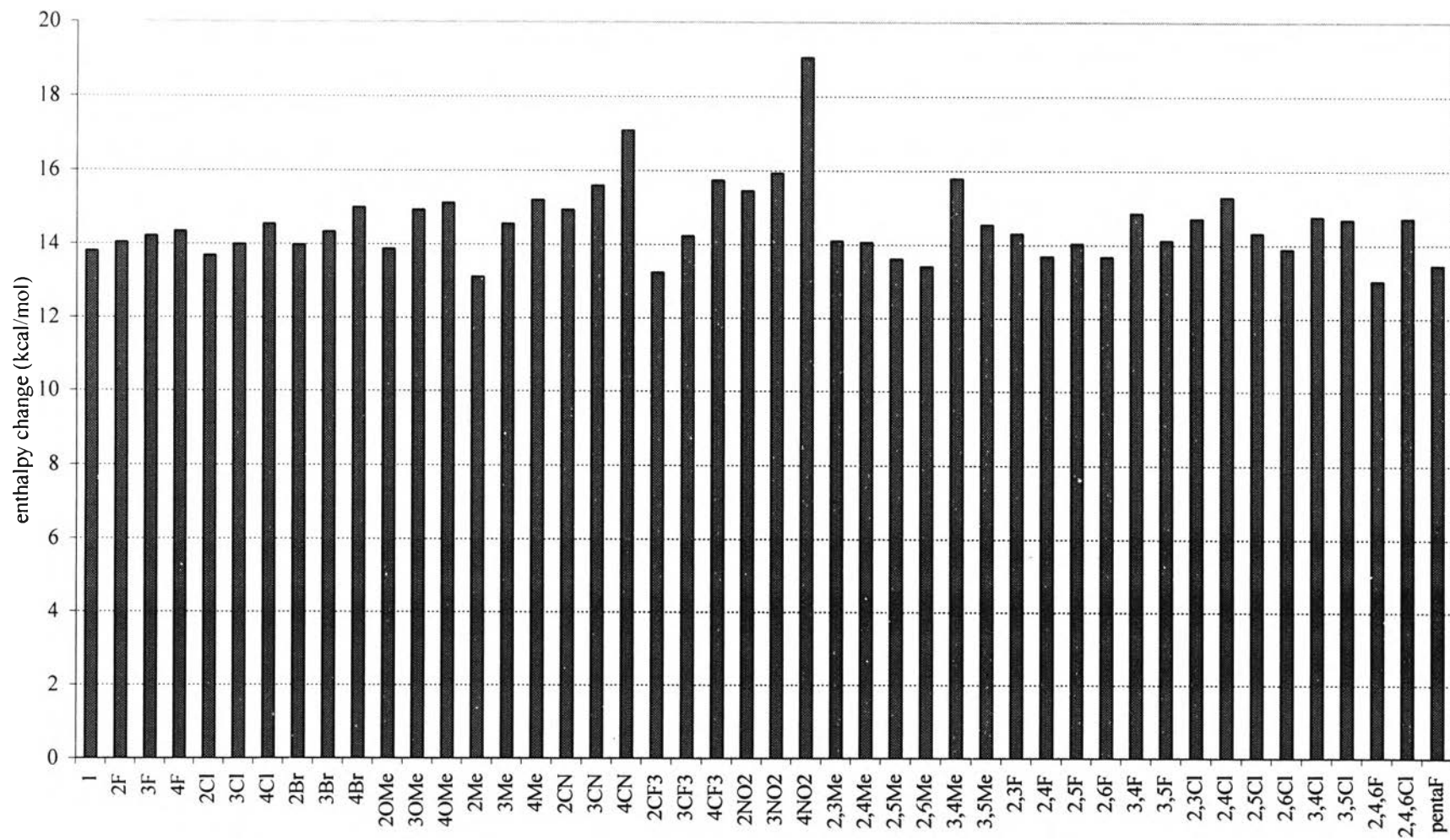
Figure 4.14   Enthalpy change (-ΔH_2, kcal/mol) of the more retained enantiomers of phenoxy acid methyl esters on BSiAc column obtained from van't Hoff approach ($\bar{x}$ = 14.54; SD = 1.06).
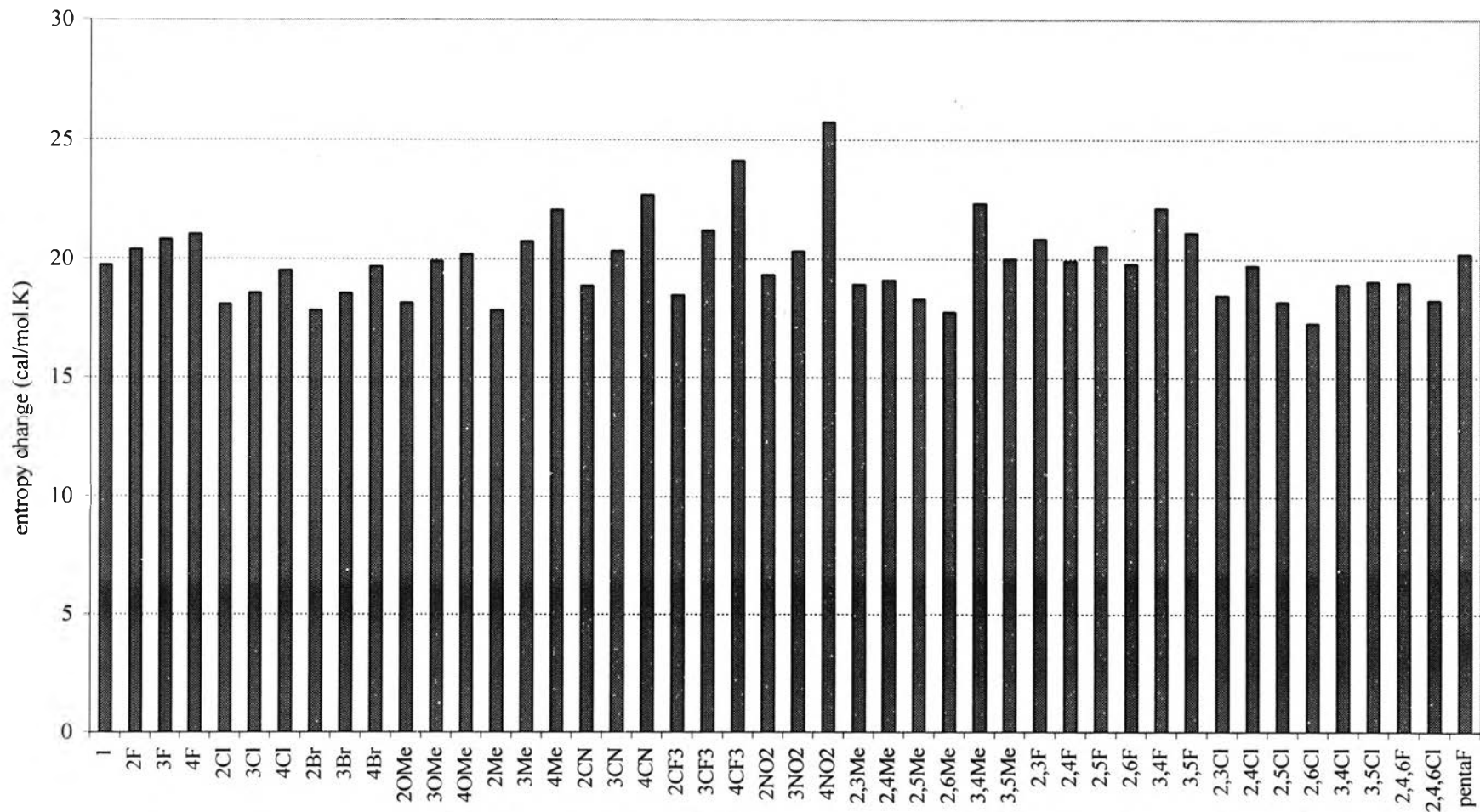
Figure 4.15 Entropy change ($-\Delta S_2$, cal/mol·K) of the more retained enantiomers of phenoxy acid methyl esters on BSiAc column obtained from van't Hoff approach ($\bar{x}$ = 20.04; SD = 1.95).

4.4.2 Enthalpy difference (-Δ(ΔH)) and entropy difference (-Δ(ΔS))

The difference in enthalpy values (-Δ(ΔH)) is a direct measure of the chiral discrimination energies between the enantiomers and the stationary phase, whereas the -Δ(ΔS) values reflected the entropy differences between a pair of enantiomers during chiral recognition process. The -Δ(ΔH) and -Δ(ΔS) values studied in this research calculated from the difference in ΔH and ΔS of two enantiomers through van't Hoff plot of ln k' versus 1/T since the relationship between ln α and 1/T occasionally exhibited curvatures in temperature range examined. This possibly resulted from various interaction types associated with the formation of an inclusion complex between analyte and chiral stationary phase, such as dipole-dipole, dipole-induced dipole, van der Waals, hydrophobic, hydrogen bonding and etc.[38]. The deviation from linearity may be an indicator of a change in the dominant interaction or a change of interaction mechanism between an analyte and a stationary phase during the temperature change. The -Δ(ΔH) and -Δ(ΔS) values of phenoxy acid methyl ester enantiomers on BSiMe and BSiAc columns were displayed on figures 4.16-4.19. The -Δ(ΔS) values of the same enantiomers on BSiMe and BSiAc columns yielded similar trends as their corresponding enthalpy difference values, -Δ(ΔH). Therefore, discussion regarding enantioseparation on both columns will refer to -Δ(ΔH) values only.

In this research, methyl 2-phenoxypropanoate (1) was regarded as a reference analyte and the influence of substituent type and position on enantioseparation were systematically explored and discussed.

The -Δ(ΔH) values representing the enantioseparation of analytes on BSiMe and BSiAc columns are definitely varied, suggesting different enantioselectivities of both phases. Approximately 35 and 9% of all analytes on BSiMe and BSiAc columns, respectively, exhibited higher enantioseparation than a reference analyte (1). Detailed discussion relating to thermodynamic values is divided into two parts according to the analyte structure as followed.
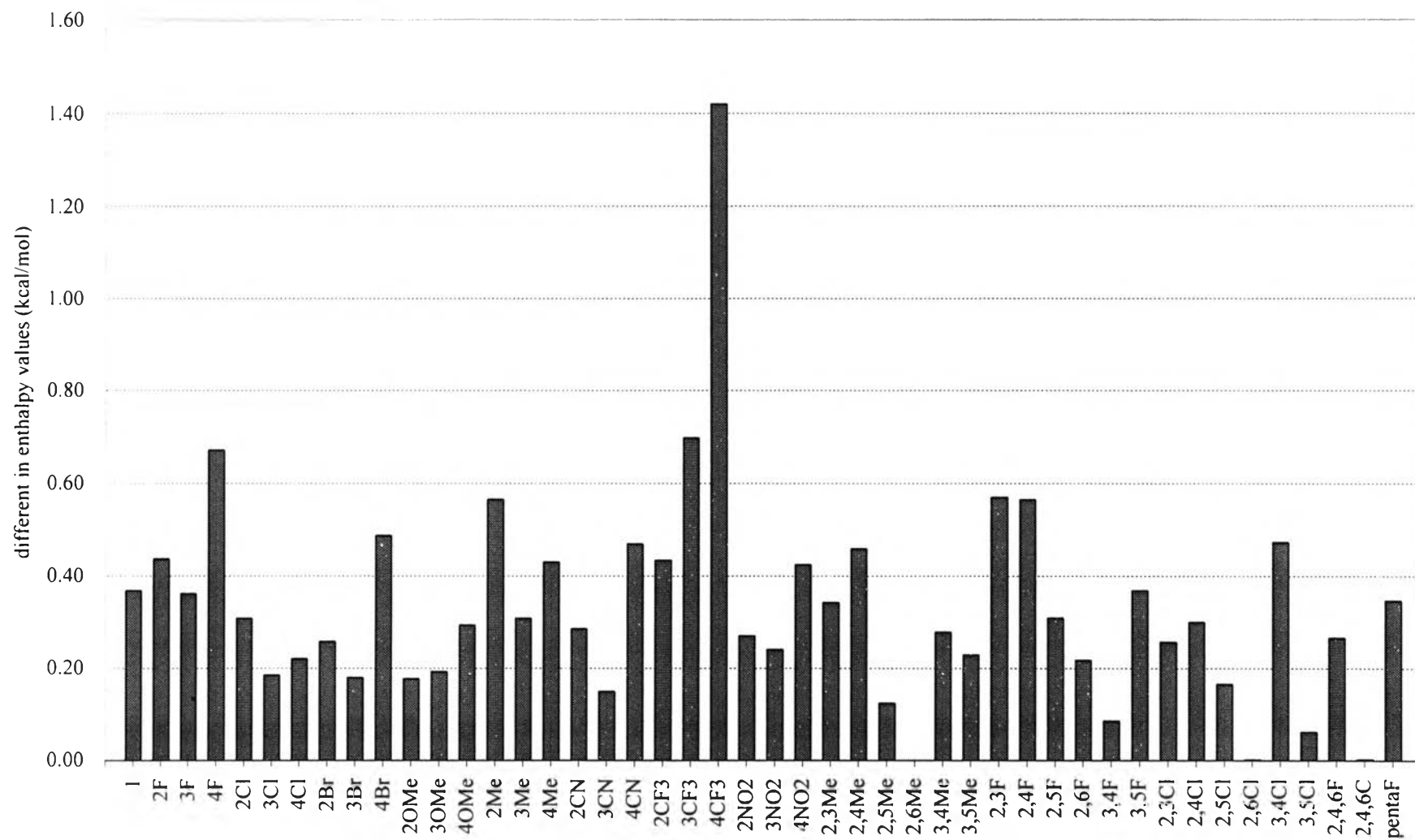
Figure 4.16   Difference in enthalpy values (-Δ(ΔH), kcal/mol) of the enantiomers of phenoxy acid methyl esters on BSiMe column.
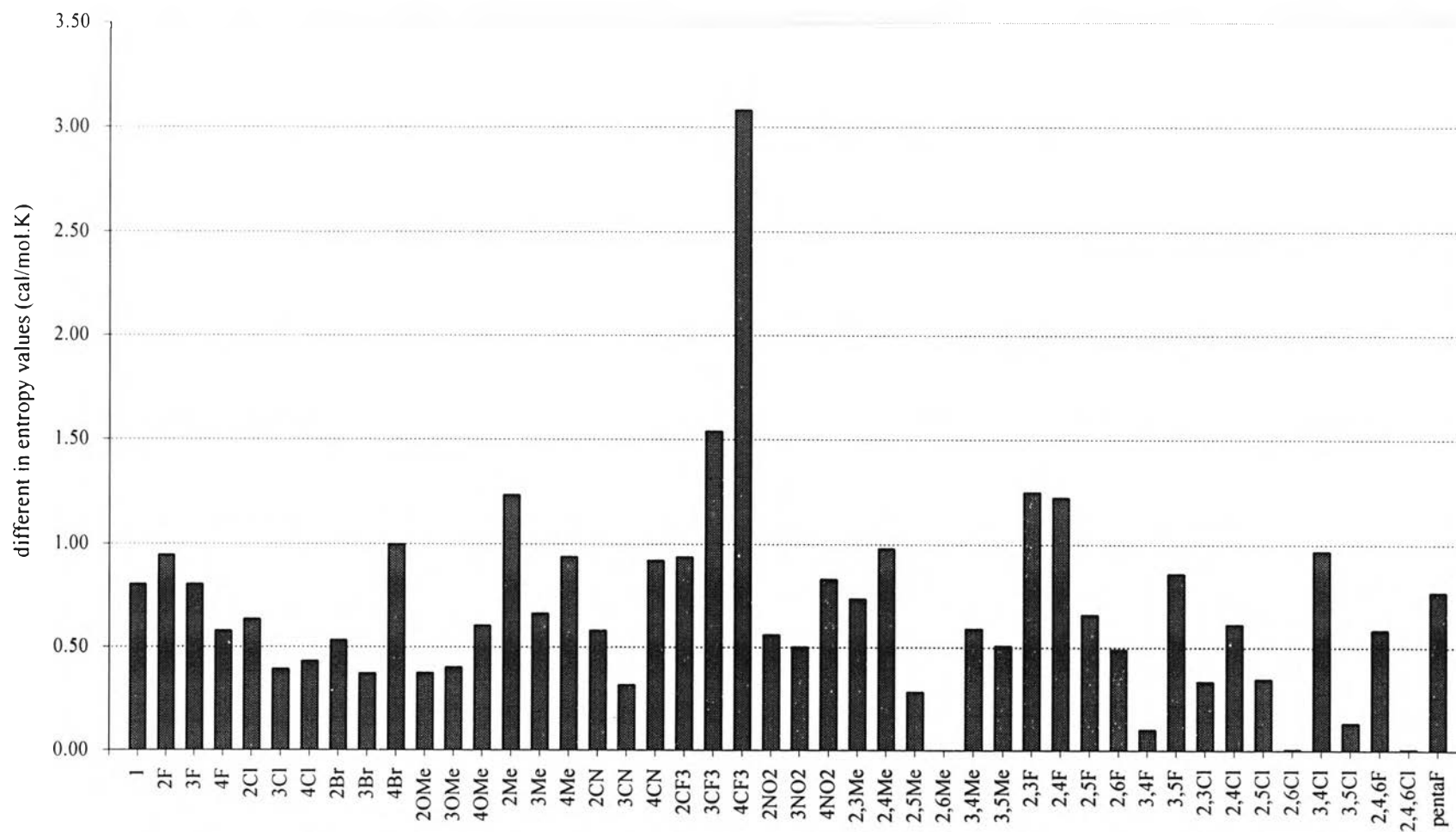
Figure 4.17 Difference in entropy values ($-\Delta(\Delta S)$, cal/ mol·K) of the enantiomers of phenoxy acid methyl esters on BSiMe column.
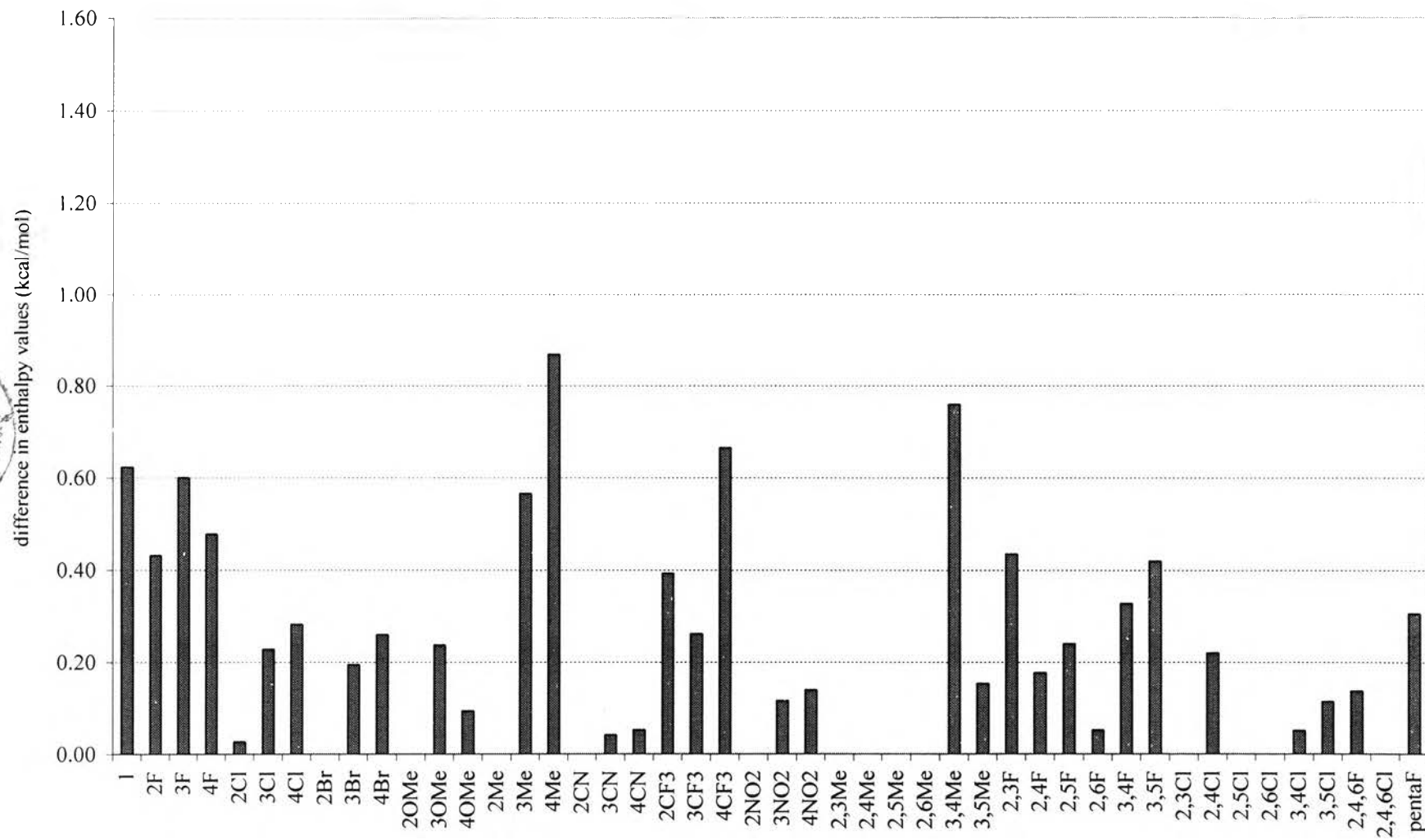
Figure 4.18 Difference in enthalpy values (-Δ(ΔH), kcal/mol) of the enantiomers of phenoxy acid methyl esters on BSiAc column.
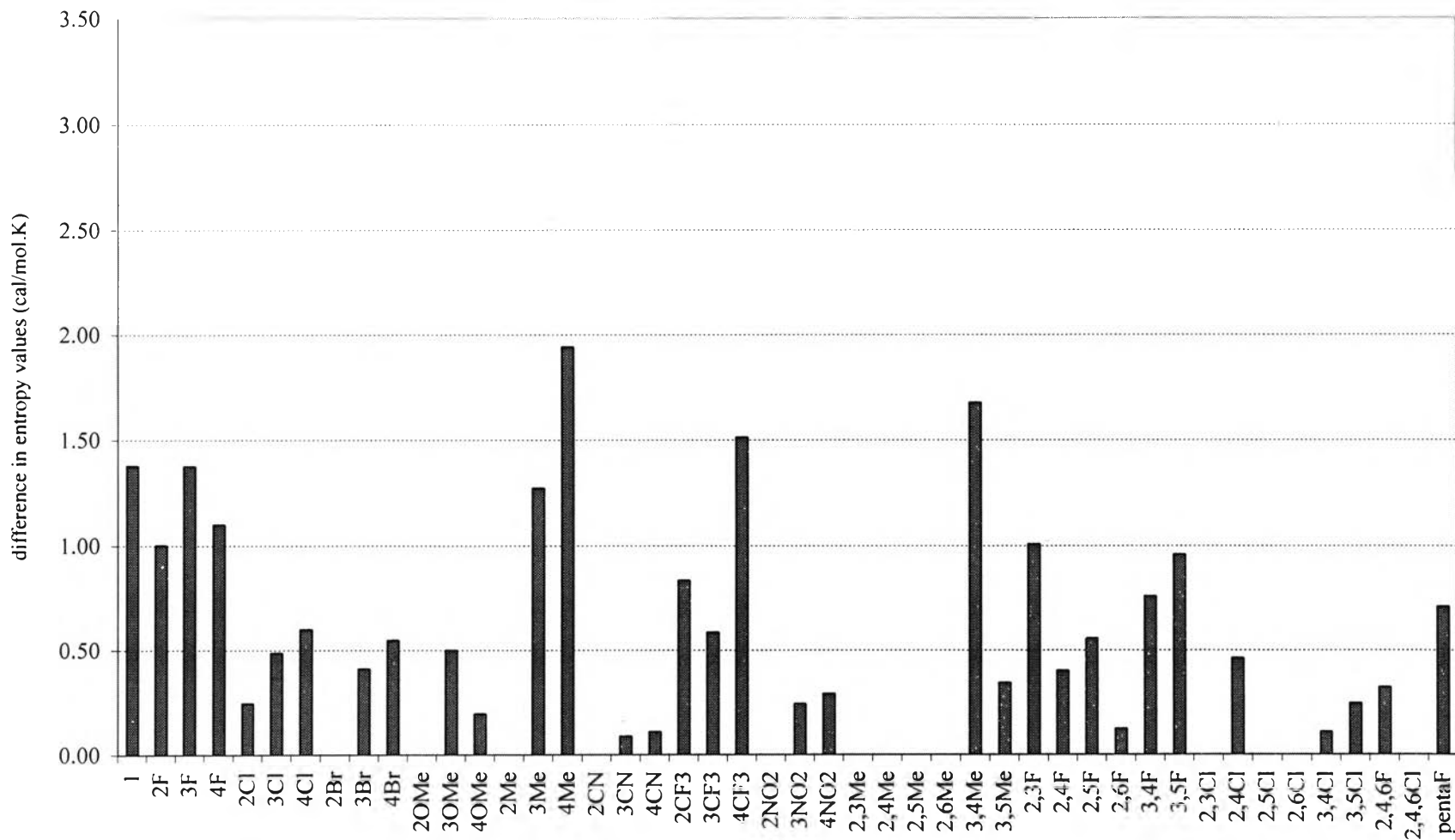
Figure 4.19 Difference in entropy values (-$\Delta(\Delta S)$, cal/ mol · K) of the enantiomers of phenoxy acid methyl esters on BSiAc column.

Series 1: Phenoxy acid methyl esters with mono-substitution on the aromatic ring

Racemic phenoxy acid methyl esters in series 1 are derivatives of methyl 2-phenoxypropanoate with mono-substitution on an aromatic ring, as shown below. The substituent types are fluoro, chloro, bromo, methoxy, methyl, cyano, trifluoromethyl and nitro at *ortho-*, *meta-* or *para-*position.

R = F, Cl, Br, OMe, Me, CN, CF_3, NO_2
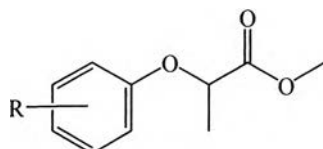
Considering the effect of substituent position on BSiMe column, mono-substitution at *para-*position on the aromatic ring is likely to promote the enantiorecognition as seen from their high -$\Delta(\Delta H)$ values, except for **4Cl** and **4OMe** (figures 4.16 and 4.20). Among *para-*substituted analytes, **4CF$_3$** exhibited the highest -$\Delta(\Delta H)$ and -$\Delta(\Delta S)$ values and gave the best separation among all tested analytes. While the substitution at *meta-*position seems to diminish the enantioseparation of analytes, except for trifluoromethyl (**3CF$_3$**). These results demonstrated the importance of substitution position. However, type of substituent also plays an important role in enantioseparation as well, as seen from trifluoromethyl-substituted analytes. The -$\Delta(\Delta H)$ values of trifluoromethyl-substituted esters decreased in the order of **4CF$_3$** >> **3CF$_3$** > **2CF$_3$**. The relationships between ln α versus 1/T of these three esters are shown in figure 4.21. It is clear that **4CF$_3$** has superior enantioselectivity (α) at all temperatures. Additionally, **4CF$_3$** also showed the highest slope, indicating that the enantioseparation of **4CF$_3$** could be simply improved with a slight decrease in temperature (figure 4.22).
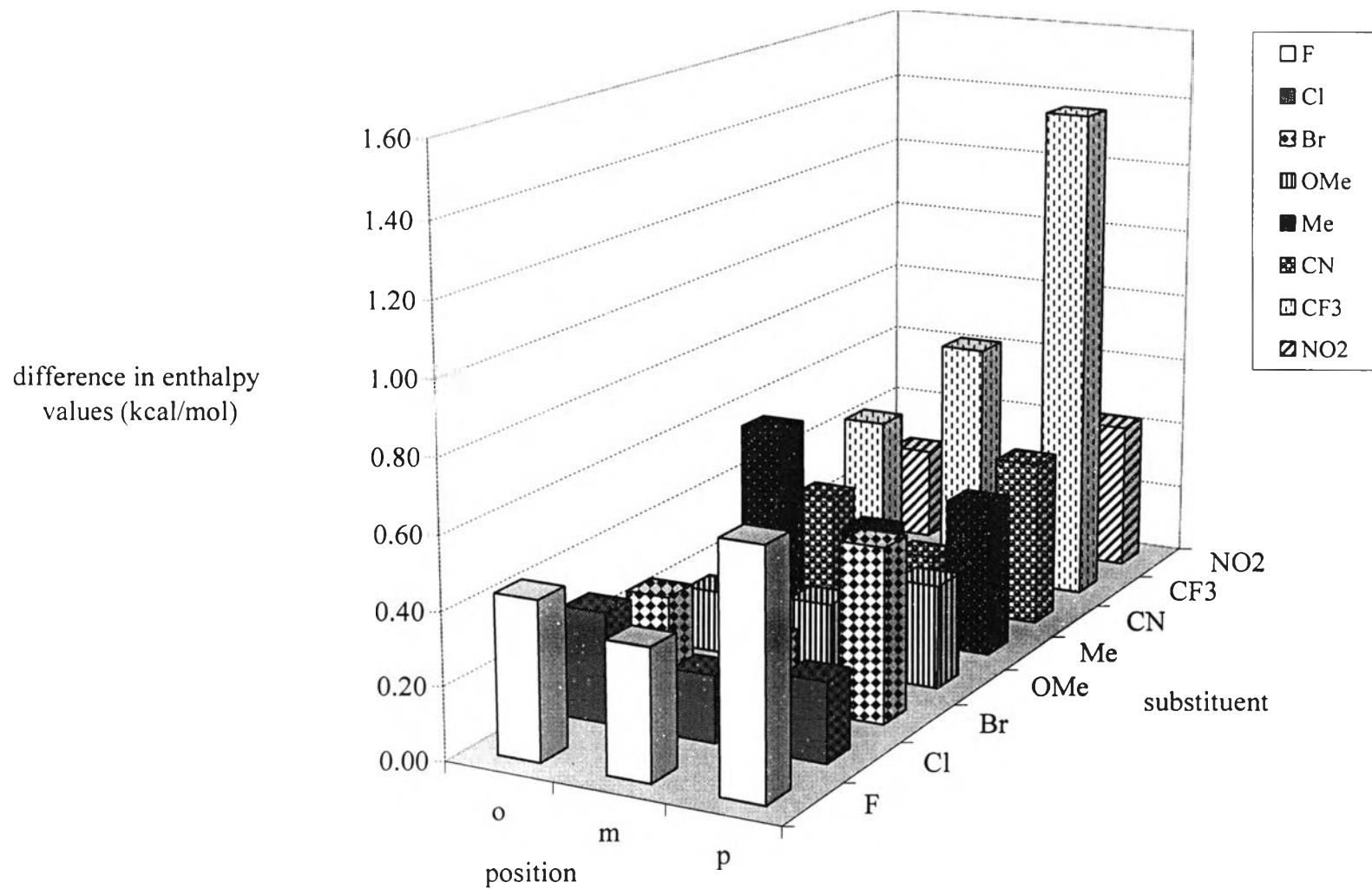
Figure 4.20 Difference in enthalpy values (-$\Delta(\Delta H)$, kcal/mol) of the enantiomers of mono-substituted methyl 2-phenoxypropanoate derivatives on BSiMe column.
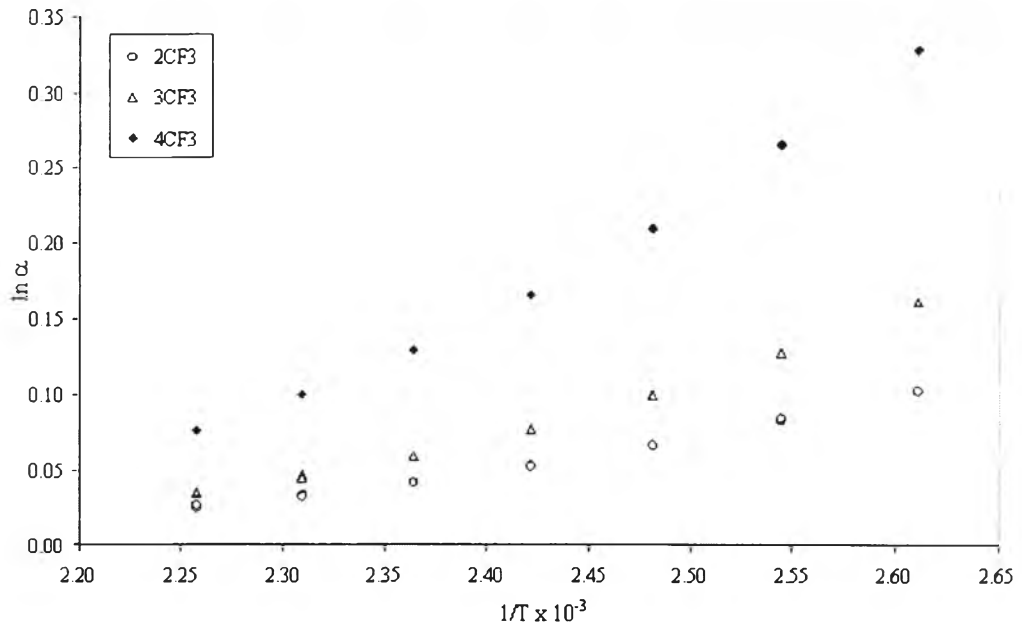
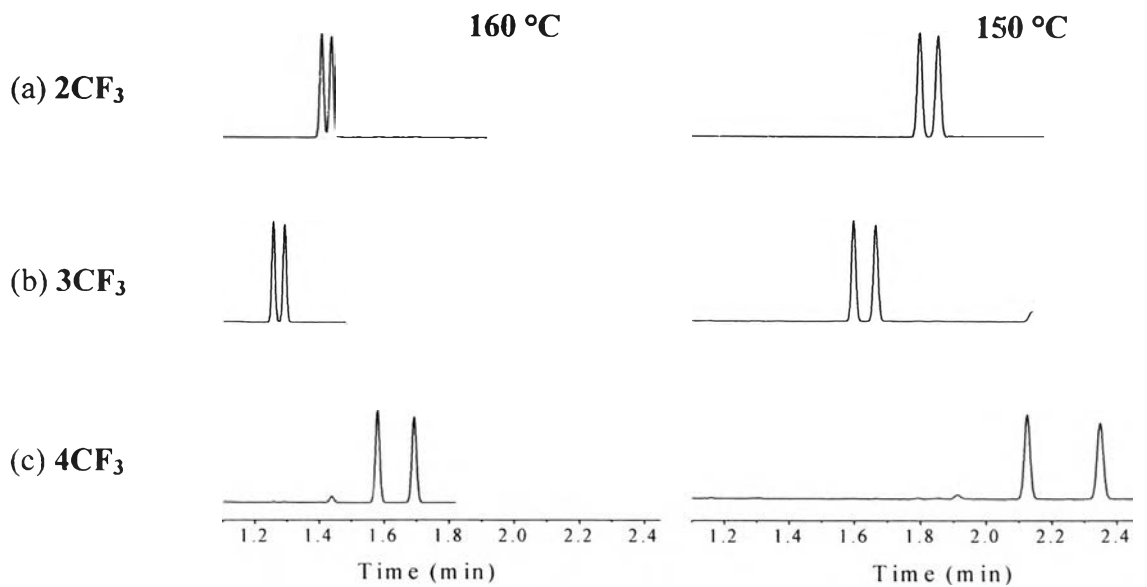**Figure 4.21** Plots of ln α versus 1/T of **2CF₃**, **3CF₃** and **4CF₃** on BSiMe column.

Figure 4.22 Chromatograms of (a) **2CF₃**, (b) **3CF₃**, (c) **4CF₃** on BSiMe column at (left) 160 °C and (right) 150 °C.

Among all *para*-substituted phenoxy acid methyl esters, -$\Delta(\Delta H)$ values decreased in the order of **4CF₃** > **4F** > **4Br** > **4CN** > **4Me** > **4NO₂** > **4OMe** > **4Cl**. When the aromatic proton was replaced by small or highly electronegative substituent, e.g. fluoro and trifluoromethyl, the enantioseparation was enhanced. Nevertheless, -$\Delta(\Delta H)$ values of **4CF₃** is two times greater than that of **4F**. In case of halogen-substituted analytes, it was observed that -$\Delta(\Delta H)$ values of *ortho-* and *meta-* substituted esters decreased in the order of F > Cl > Br, according to the decreasing electronegativity of substituent ($EN_F = 4.0$, $EN_{Cl} = 2.8$, $EN_{Br} = 2.7$ [44]) and to the increasing of substituent size ($r_F = 131$ pm, $r_{Cl} = 181$ pm, $r_{Br} = 196$ pm [45]). On the other hand, -$\Delta(\Delta H)$ values of *para*-substituted esters did not follow the same trend and were in the order of **4F** > **4Br** >> **4Cl**.

On BSiAc phase, where cyclodextrin substituents were changed from nonpolar methyl to polar acetyl groups, the enantioseparation of phenoxy acid methyl esters were evidently different from that on BSiMe column. For the reference analyte (1), the -$\Delta(\Delta H)$ value on BSiAc is almost twice the value on BSiMe. Nonetheless, it was surprising to note that about 90% of substituted analytes had -$\Delta(\Delta H)$ values lower than that of 1 (figure 4.18). Substituents with bulky and strong electron withdrawing groups (e.g. cyano, nitro) at any position effectively decrease the -$\Delta(\Delta H)$ values of analytes. Interestingly, **4Me** and **4CF₃** displayed higher -$\Delta(\Delta H)$ values than 1 and, additionally, **4Me** offered the greatest enantioseparation on BSiAc column.

Considering the effect of substituent position, BSiAc column showed poor or no enantiodifferentiation for *ortho*-substituted analytes, except for **2F** and **2CF₃** (figures 4.23 and 4.24). These were probably caused by the steric hindrance of the *ortho*-substituted position along with the larger acetyl groups that obstructed the interaction between analytes and BSiAc phase. On the contrary, most *meta*-substituted analytes displayed improved enantioseparation on BSiAc phase compared to BSiMe phase, especially for **3F** and **3Me**, and the highest improvement was observed with **3Me**.
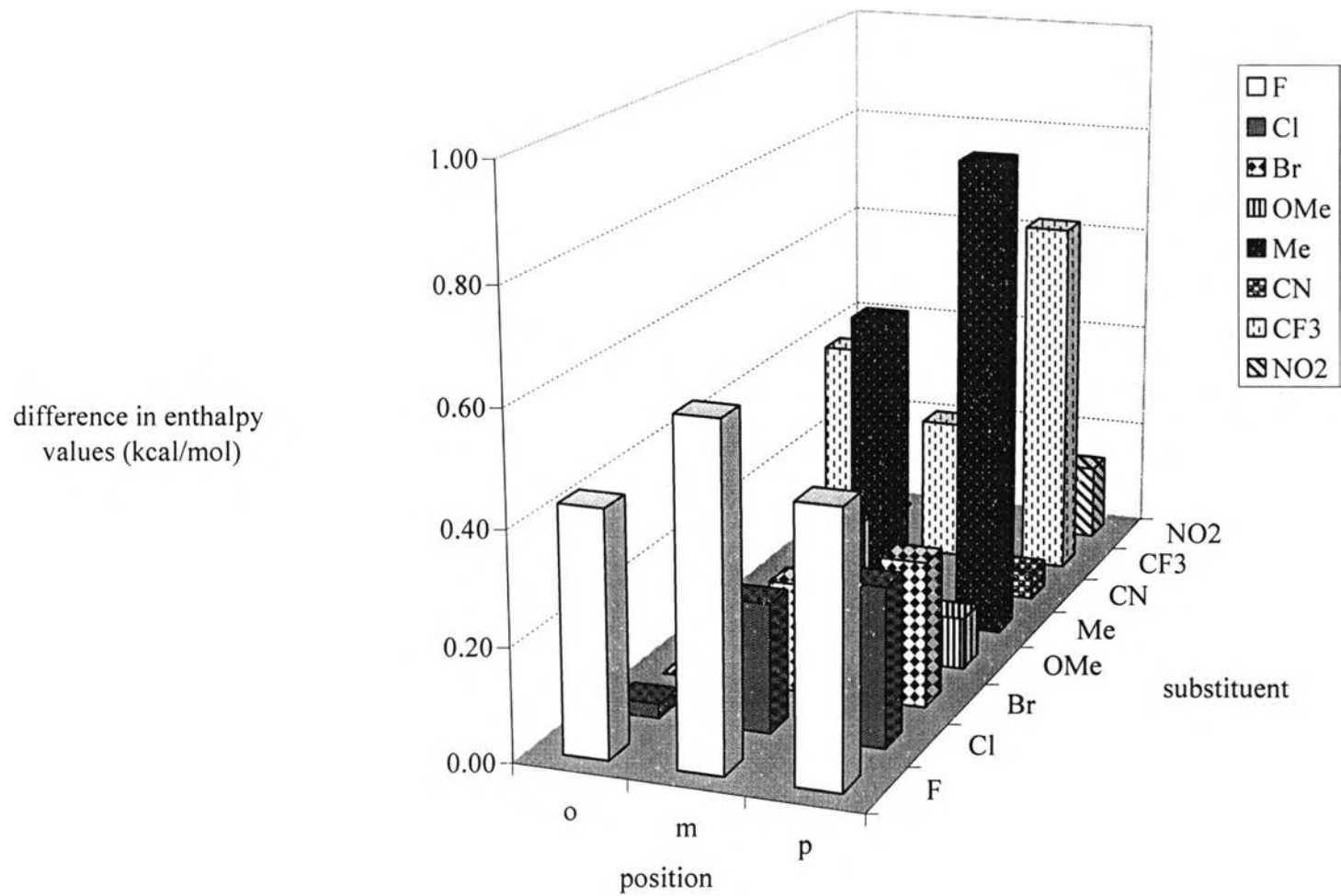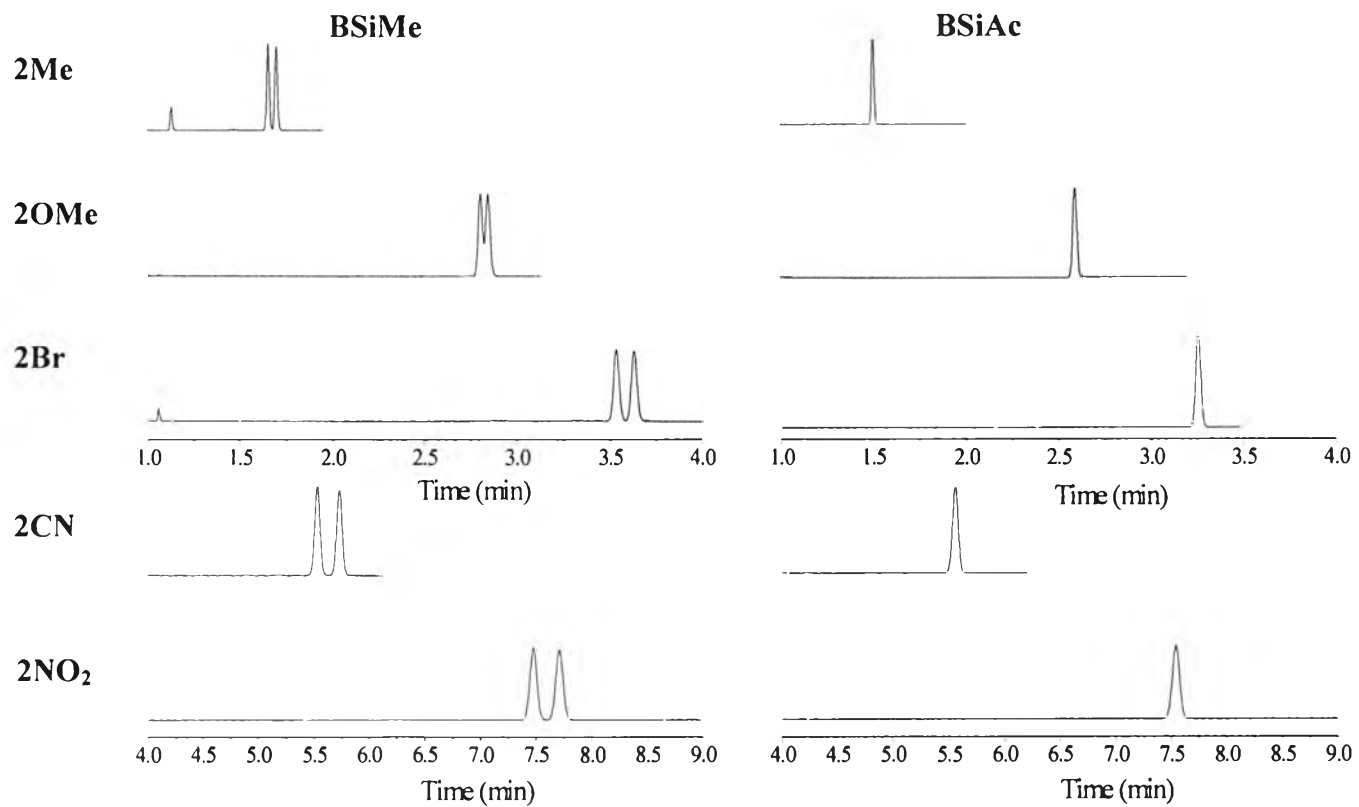
Figure 4.23 Difference in enthalpy values (-Δ(ΔH), kcal/mol) of the enantiomers of mono-substituted methyl 2-phenoxypropanoate derivatives on BSiAc column.

Figure 4.24 Chromatograms of **2Me, 2OMe, 2Br, 2CN, 2NO₂** on BSiMe and BSiAc columns at 160°C.

The similar effect of *meta*-substitution on enantioseparation on BSiAc was observed in the previous work as well. Yanjinda [46] reported that the enantioseparation of *meta*-substituted styrene oxide derivatives on BSiAc were greater than that on BSiMe; however, the *ortho*-and *para*-substituted analytes exhibited the adverse tendency. The relationship between ln α versus 1/T of **3Me** on both chiral columns was shown in figure 4.25. It can be seen that temperature has a strong effect toward **3Me** on BSiAc, but shows a slight effect on BSiMe.

At temperatures below 150 °C, separation selectivity for the enantiomers of **3Me** on BSiAc is better than that on BSiMe, but above 150 °C the opposite is observed. The enantioseparation of **3Me** on both chiral columns at various temperatures are shown in figure 4.26. These results also demonstrated the influence of type of CD substituent on enantioseparation and separation mechanism of phenoxy acid methyl esters.
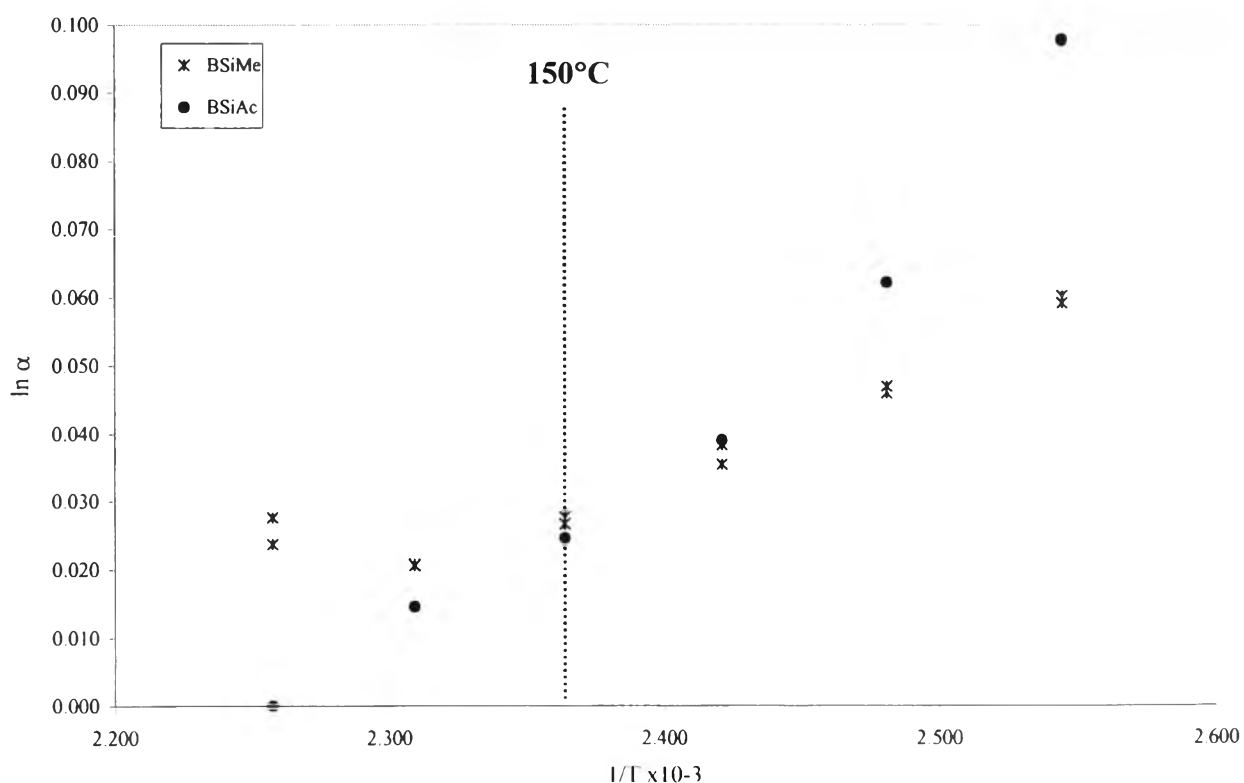

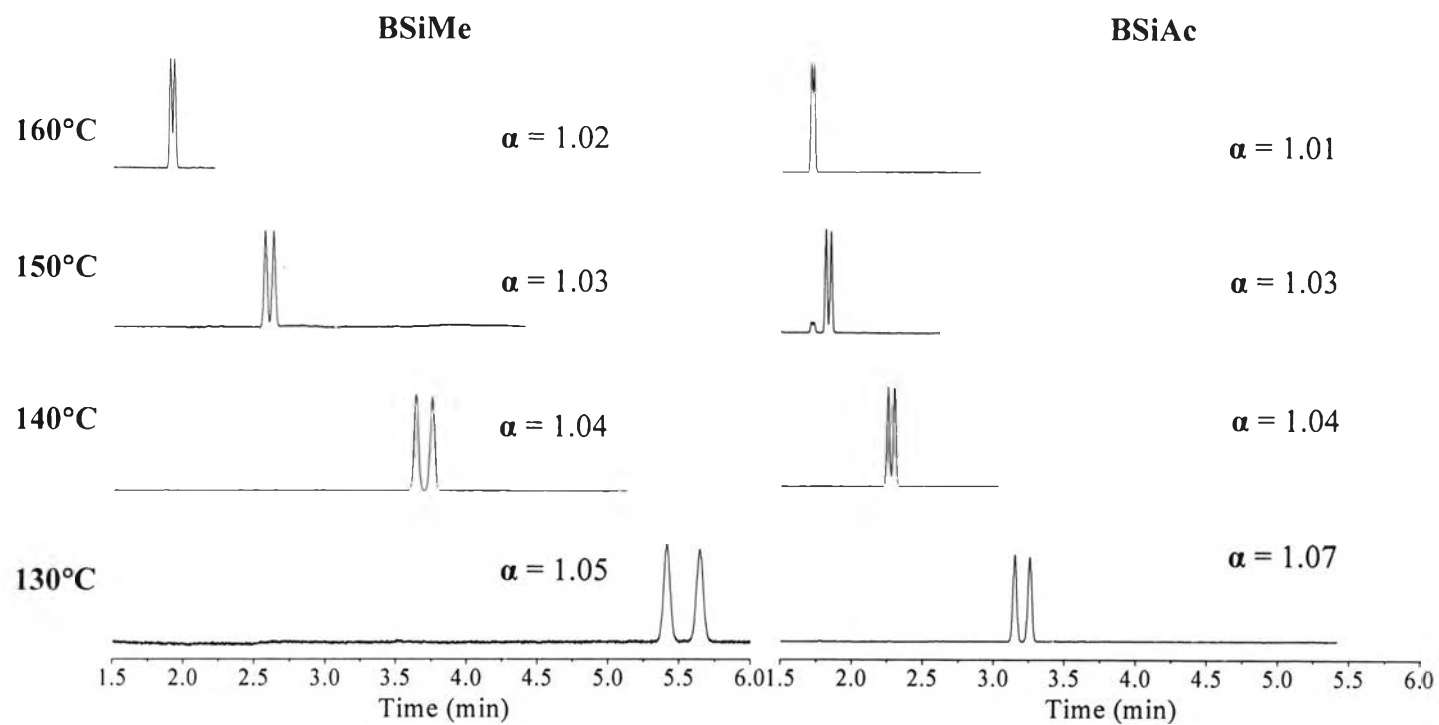**Figure 4.25**   Plots of ln α versus 1/T of **3Me** on BSiMe and BSiAc columns.

Figure 4.26 Chromatograms of **3Me** on BSiMe and BSiAc columns at 160°C, 150°C, 140°C and 130°C.

Series 2: Phenoxy acid methyl esters with di-substitution on the aromatic ring

Racemic analytes in this series are composed of phenoxy acid methyl esters derivatives with dimethyl-, difluoro-, and dichloro-substitutions at different positions of the aromatic ring as shown below.
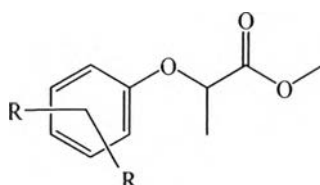
R = F, Cl, Me

The $-\Delta(\Delta H)$ values representing the enantioseparation of di-substituted phenoxy acid methyl esters on BSiMe and BSiAc columns were illustrated in figures 4.27-4.28. The $-\Delta(\Delta S)$ values also exhibited similar trends as their corresponding $-\Delta(\Delta H)$ values on each column as seen on figures 4.17 and 4.19.

On BSiMe column, fluoro-substituted analytes still displayed high $-\Delta(\Delta H)$ values and all difluoro-substituted analytes could be successfully separated on this stationary phase. The two greatest enantioseparations were observed with **2,3F** and **2,4F**. These results confirm that fluoro substitution certainly plays a key role on enantioseparation as those observed in *series 1*. Additionally, lower $-\Delta(\Delta H)$ values were observed for methyl- and chloro-substituted analytes. Among 18 di-substituted phenoxy acid methyl esters, **2,6Cl** and **2,6Me** could not be separated, whereas **2,6F** was efficiently resolved on BSiMe phase. This result demonstrated that steric substituents (chloro and methyl) hindered the interaction between analytes and cyclodextrin derivative and, thus, no enantiorecognition was observed (figure 4.29).
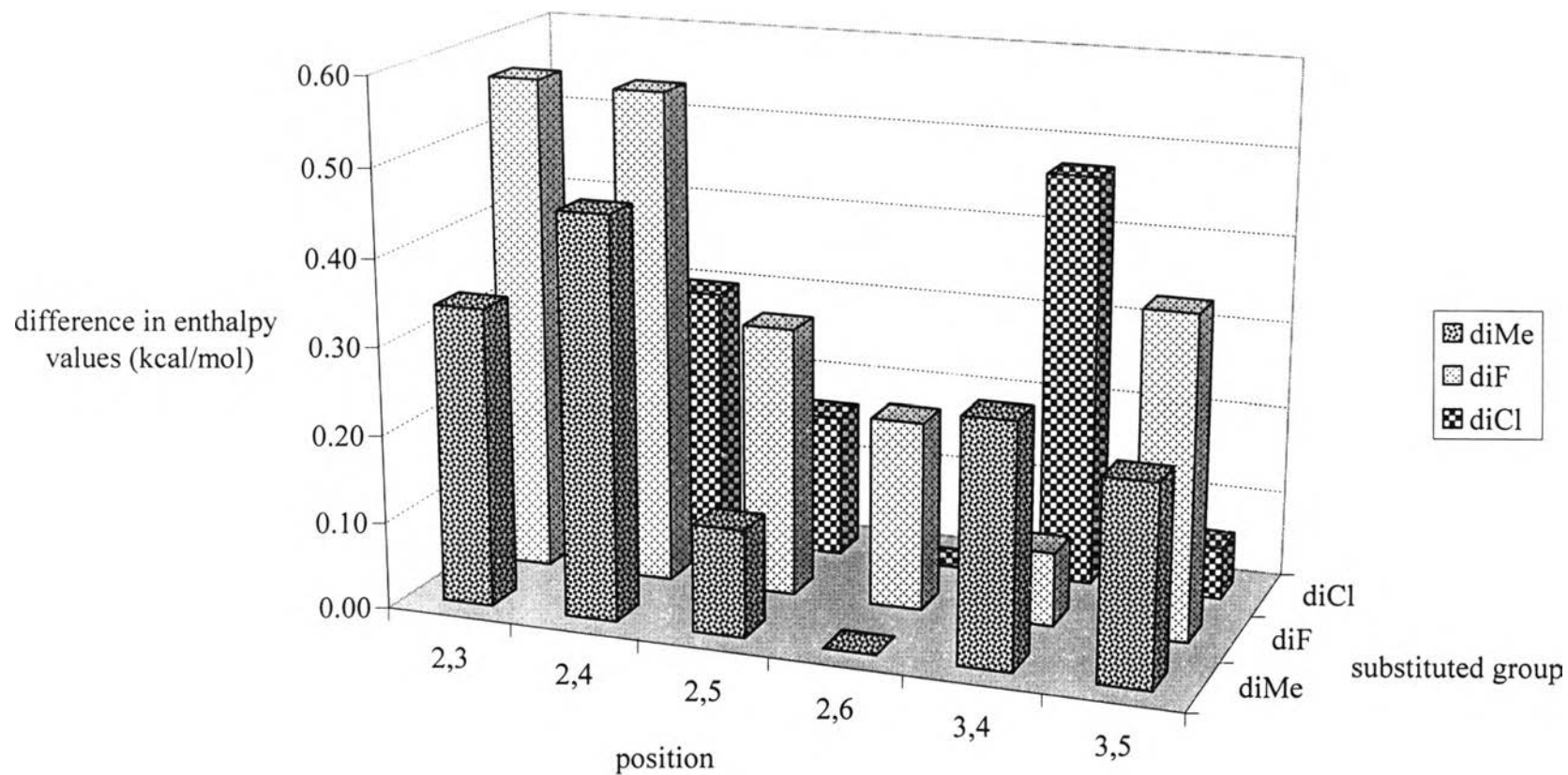
Figure 4.27 Difference in enthalpy values ($-\Delta(\Delta H)$, kcal/mol) of the enantiomers of di-substituted methyl 2-phenoxypropanoate derivatives on BSiMe column.
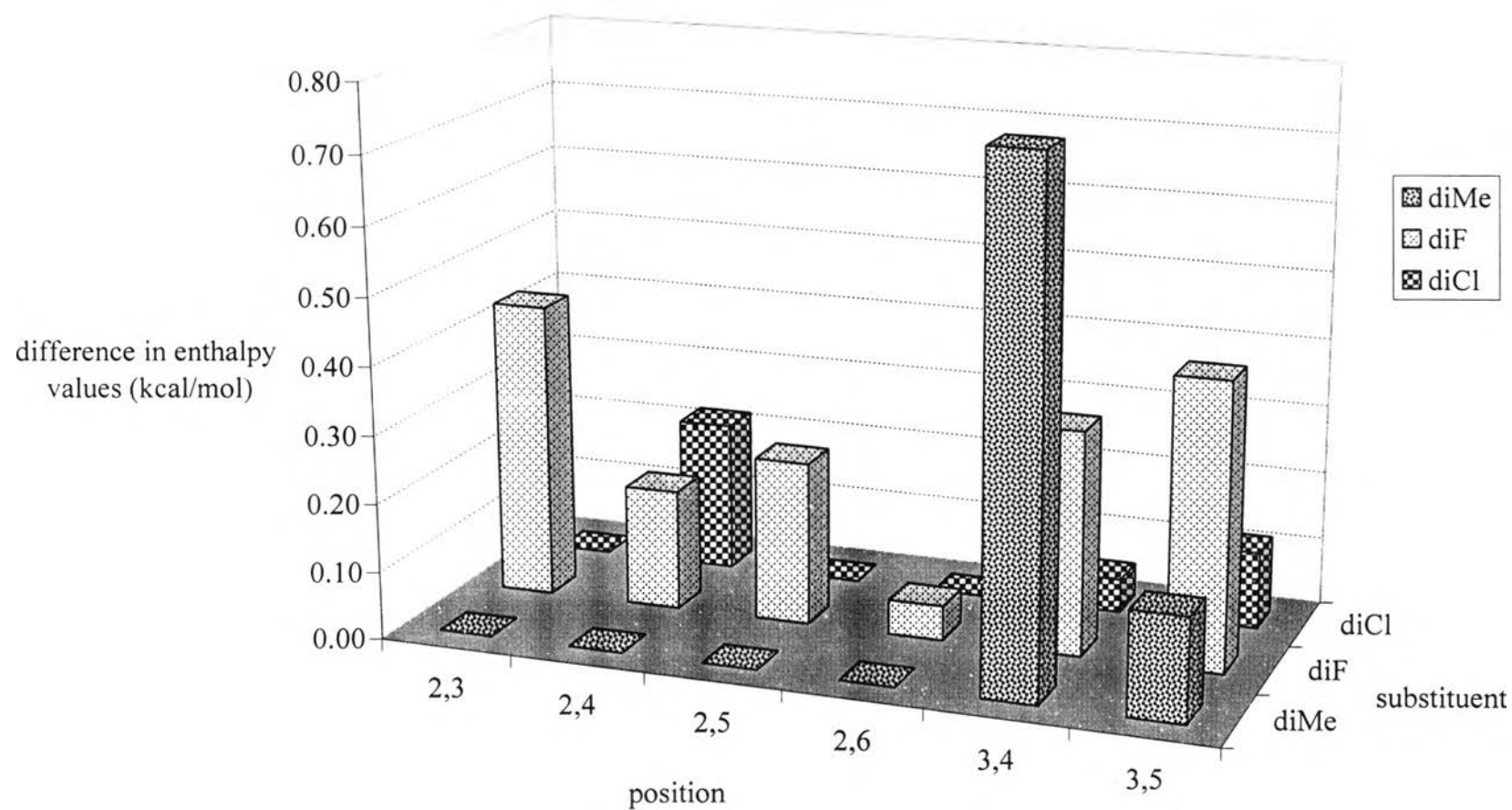
Figure 4.28 Difference in enthalpy values (-Δ(ΔH), kcal/mol) of the enantiomers of di-substituted methyl 2-phenoxypropanoate derivatives on BSiAc column.
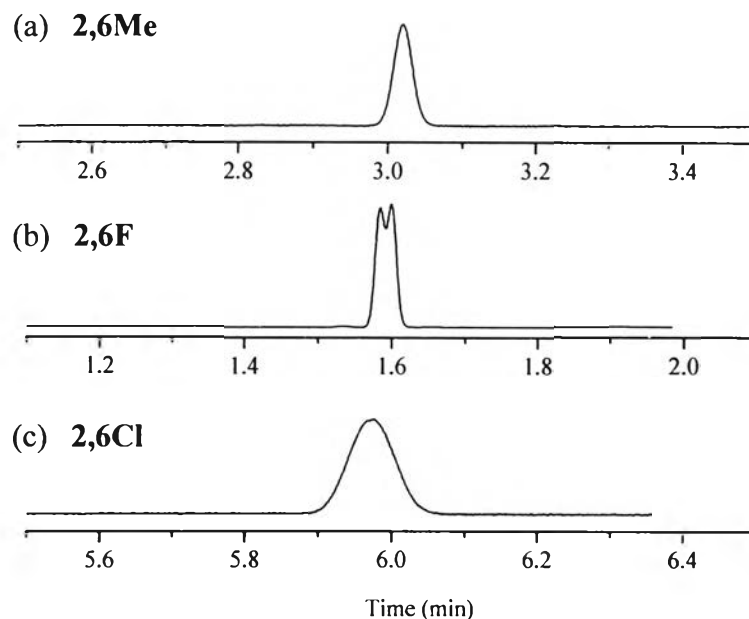
Figure 4.29 Chromatograms of (a) **2,6Me**; (b) **2,6F**; (c) **2,6Cl** on BSiMe column at 150°C.

On BSiMe, the effect of substituent position towards enantioseparation was not clear; however, a small trend could be observed. It was noticed from series 1 that *para*-substitution tended to promote the enantiorecognition. In this series, analytes containing *para*-substituent (as in 2,4- and 3,4-positions) also exhibited high -$\Delta(\Delta H)$ values, except for **3,4F**.

On BSiAc column, all di-substituted phenoxy acid methyl esters showed reduced -$\Delta(\Delta H)$ values compared to reference analyte (**1**), except for **3,4Me**. All difluoro-substituted analytes were still resolved on this phase; however, most of their -$\Delta(\Delta H)$ values were lower than those on BSiMe column, except for **3,4F**. Most of dichloro- and dimethyl-substituted analytes (**2,3Cl**, **2,5Cl**, **3,4Cl**, **2,3Me**, **2,4Me**. and **2,5Me**) that could be separated on BSiMe column were poorly resolved or not separated on BSiAc phase (figures 4.30-4.31). Nonetheless, only **3,4Me** was well separated on this phase and its -$\Delta(\Delta H)$ value was about three times its value on BSiMe phase.
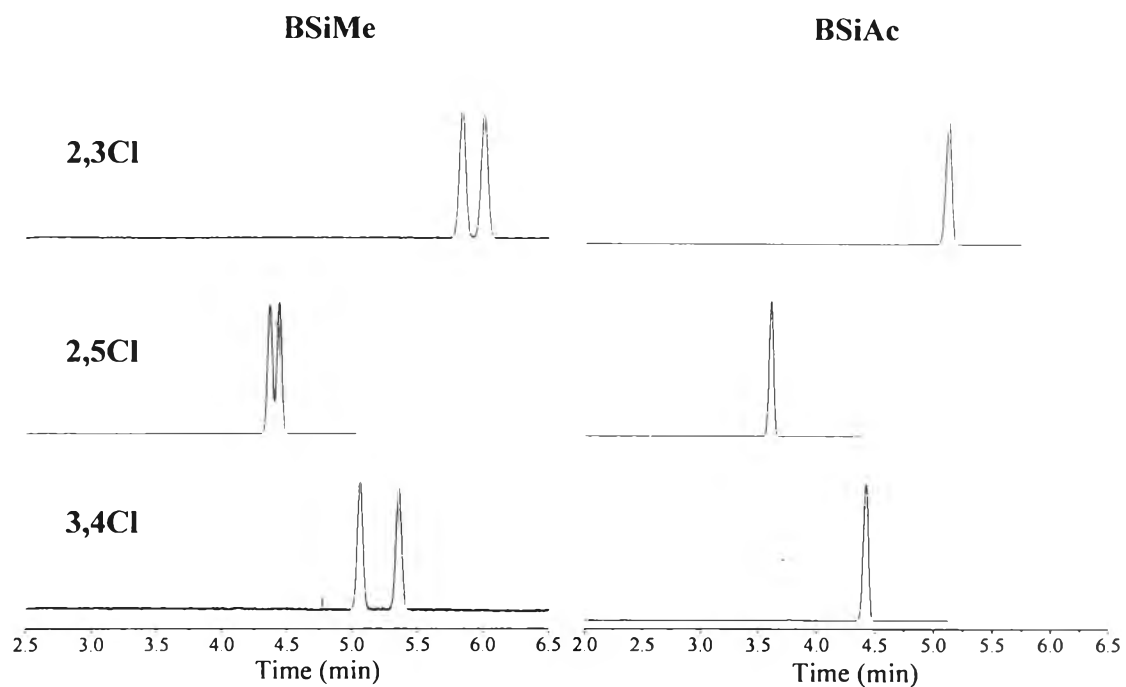
Figure 4.30 Chromatograms of **2,3Cl**, **2,5Cl** and **3,4Cl** on BSiMe and BSiAc columns at 160°C.
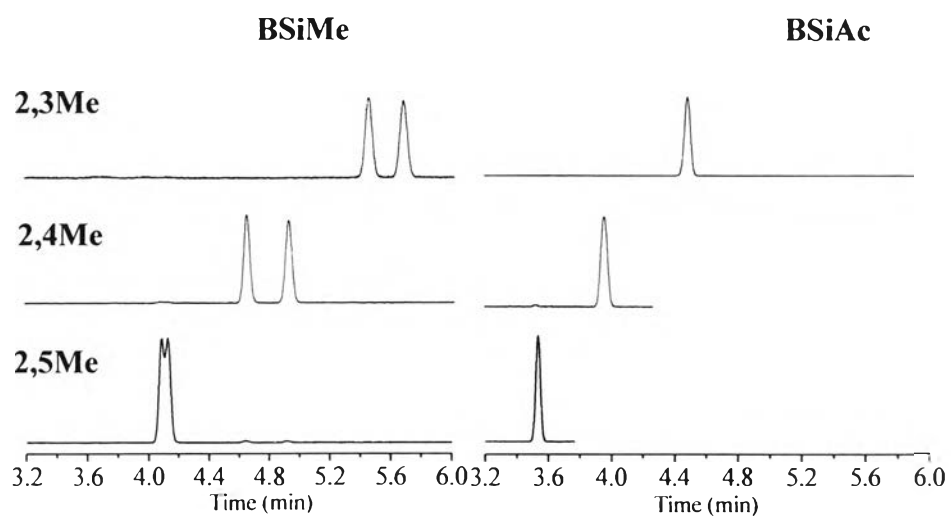

Figure 4.31 Chromatograms of **2,3Me**, **2,4Me** and **2,5Me** on BSiMe and BSiAc columns at 140°C.

This result was in agreement with superior separation of **3Me** and **4Me** on BSiAc column. It could be assumed that methyl group at *meta-* and/or *para*-position probably brought about appropriate analyte conformation for interaction with BSiAc, thus improved enantiorecognition was observed. Plots of ln α versus 1/T of **3,4Me** on both chiral columns were compared in figure 4.32 and their chromatograms were illustrated in figure 4.33. It can be noted that the temperature strongly affects toward **3,4Me** on BSiAc, but shows a small effect on BSiMe. The resolution of **3,4Me** on BSiAc was better, and even within shorter analysis time.
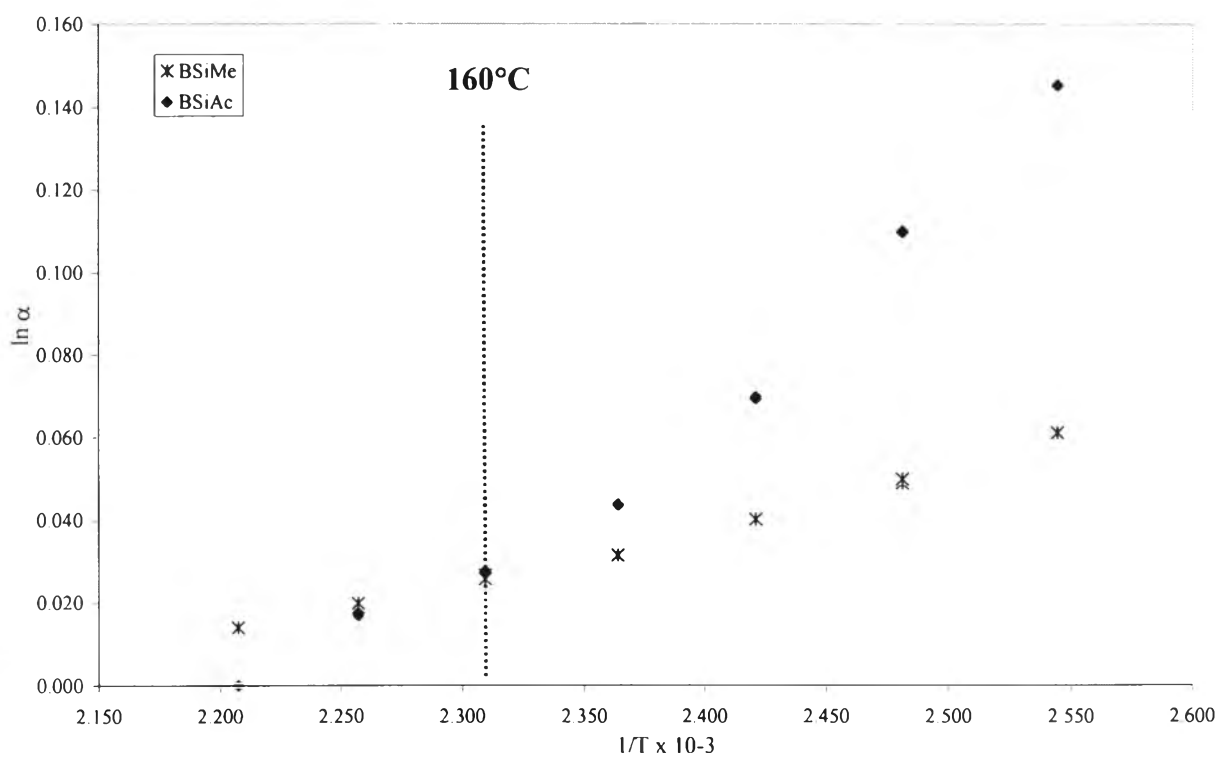

Figure 4.32   Plots of ln α versus 1/T of **3,4Me** on BSiMe and BSiAc columns.

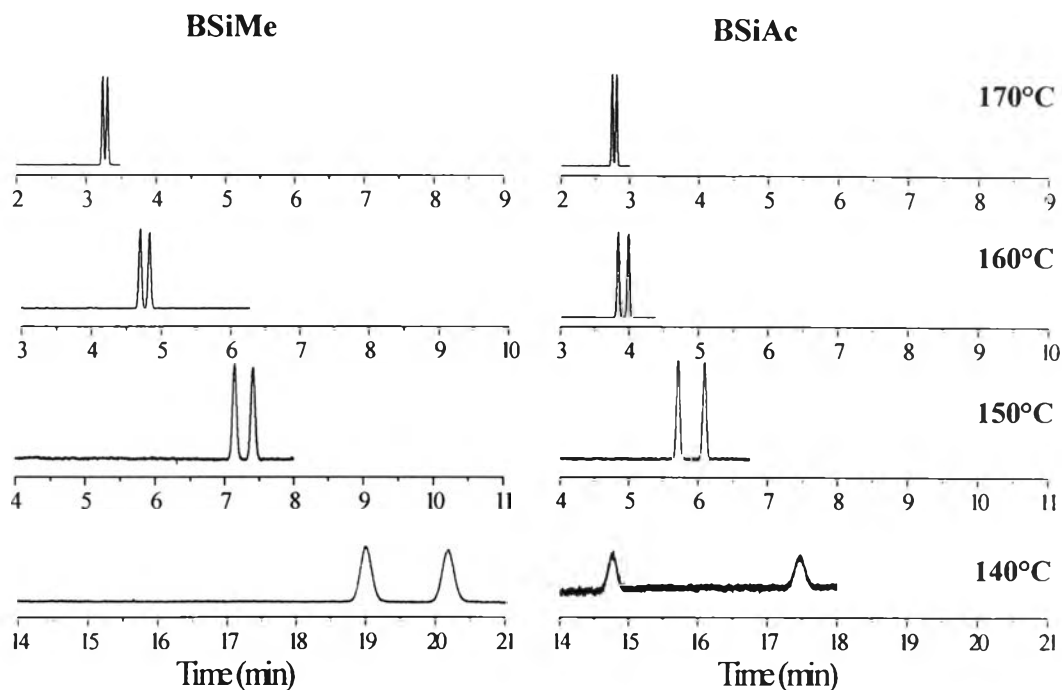**BSiMe**   **BSiAc**

Figure 4.33   Chromatograms of **3,4Me** on BSiMe and BSiAc columns at 170°C,
160°C, 150°C and 140°C.

The preliminary investigation of the influence of number of aromatic-
substituent towards enantioseparation on both chiral stationary phases was also
performed. It was observed that trichloro-substituted ester, **2,4,6Cl**, showed no
separation on both columns. This result corresponded with previous report by
Weber *et al.* [9] that examined the influence of number of substitutent on the
enantioselectivity of phenoxypropionates with permethylated β-cyclodextrin stationary
phase. It was indicated that increasing number of chlorine atoms could decrease
electron density in the aromatic system resulting in poor enantioselectivity; particularly
the enantioselectivity of methyl 2-(2′,4′,5′-trichlorophenoxy)propionate was three fold
lower than that of methyl 2-(2′-methyl-4′-chlorophenoxy) propionate. For trifluoro-
substituted ester, lower -Δ(ΔH) value of **2,4,6F** than that of reference **1** was also
noticed on both columns. The replacement of all aromatic protons of **1** with fluorine
atoms, as in **pentaF**, gave only a small decrease in -Δ(ΔH) value on BSiMe column.

However, comparing to reference **1**, **pentaF** showed a significant reduction in -$\Delta(\Delta H)$ value on BSiAc column.Unfortunately, other tri-, tetra- and penta-substituted analytes and their isomers are not available; thus, a common trend on the effect of number of aromatic-substituent on enantioseparation could not be proposed.