Research Article

Free Energy Contribution to Gas Chromatographic Separation of Petroselinate and Oleate Esters

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The ease of separation by gas chromatography between petroselinic and oleic acids depends on the alcohol moieties of their esters. The esters of higher molecular weight alcohols tend to be better separated on a 90%-biscyanopropyl-10%-cyanopropylphenyl polysiloxane capillary column (30 m × 0.25 mm i.d.). By analysis of free energies contribution from different parts of the molecules, it is tentatively concluded that the interaction between the double bond and the column stationary phase is interfered by the bulky alkyl group, and it is the major driving force for the separation of the two fatty acids.

1. Introduction

Petroselinic acid (18:1Δ6cis) is a major fatty acid in the seed oils of most of the members of the Apiaceae (Umbelliferae), Araliaceae, Garryaceae species, Acer (Aceraceae), and Geraniaceae [1–4]. Petroselinic acid is an interesting oleochemical for food, cosmetics, and pharmaceutical industries [5]. Coriander seed oil, which contained about 70% petroselinic acid, showed a significant hypolipidemic effect on animals [6]. High petroselinic acid diets found to lower the n-6 long-chain fatty acids of the phospholipids [7]. Hence, petroselinic has become more and more important in industry and human health. In 1997, Santinelli and Damiani [8] pointed out that petroselinic acid was always accompanied by some oleic acid (18:1Δ9cis), and there was no single chromatographic technique suitable for their separation and quantitation. However, methods for its separation from oleic acid have gradually been improved. Lin et al. [9] and Liu and Hammon [10] reported the separation of these two positional isomers by reversed phase liquid chromatography. Kim et al. [11] were able to separate and quantitate petroselinic and oleic acids in coriander tissues as their methyl esters by gas chromatography (GC) on a 5%-phenyl-95%-methylpolysiloxane capillary column. In addition, Thies [12] reported the use of butyl esters for the separation of these two positional isomers at 220°C with a total run time of 13 min. Thies saw good resolution of the butyl esters under these conditions, but they were not baseline separated. On the other hand, with a highly polar column (100%-cyanopropyl polysiloxane) of 50 meters, the two isomers could almost be baseline separated as their isopropyl esters [13, 14]. These two isomers in coriander seed oil could also be separated and quantitated on a 30 m of 100%-cyanopropyl polysiloxane with two steps temperature program [15], but how well they were separated was not reported. Near-baseline separations of methyl petroselinate and oleate on 100%-cyanopropyl polysiloxane columns from two different manufacturers at 180°C were reported [16]. A recent report [17] showed that methyl petroselinate and oleate could be separated on ionic liquid stationary phase [1,9-di(3-vinyl-imidazolium) nonane bis-(trifluoromethyl) sulfonyl imidate] columns, with the lengths of 12 (the microbore capillary), 30, and 100 meters.

A detailed study on the effects of alcohol chain lengths and branched chain on the separations of petroselinic and oleic acid esters was reported by Isbell [18]. Analyses were conducted on a 30 m of 100%-cyanopropyl polysiloxane column. The authors found that 2-ethyl-1-hexyl esters were
promising derivatives for quantitative analysis of the oils containing the mixture of petroselinic and oleic acids. With the 2-ethyl-1-hexyl esters, the interaction of the polar carboxyl group with the stationary phase was sterically masked. Therefore, the main interaction would be that between the small polarity differences of the olefins and the polar stationary phase of the column, which caused the separation.

However, methyl esters of these two acids could also be separated on a 100 m of 100%-cyanopropyl polysiloxane capillary column [19].

In this study, oils containing petroselinic and oleic acids are transesterified with alcohols of different chain lengths. The gas chromatographic separations of these two positional isomers are discussed in term of their standard free energy of transfer from solution to gas ($\Delta_{g}^{s}H^0$). Thus, a general knowledge of GC related to $\Delta_{g}^{s}H^0$ is briefly reviewed.

In gas-liquid chromatography, the solute (at infinite dilution) partitioning between the stationary and the mobile phases is assumed to be at equilibrium or very close to equilibrium state. Thus, the change in $\Delta_{g}^{s}H^0$ directly relates to its equilibrium constant ($K$). The $\Delta_{g}^{s}H^0$, on the other hand, can be divided into $\Delta_{g}^{s}G^0$ and $\delta_{g}^{s}G$ [20] as described in the following:

$$\Delta_{g}^{s}G^0 = \Delta_{g}^{s}H^0 + T\Delta_{g}^{s}S^0,$$

where $z$ is the number of carbon atoms. If $z = 0$, $\Delta_{g}^{s}H^0 = \Delta_{g}^{s}G^0$, that is $\Delta_{g}^{s}G^0$ is the free energy contributed from the hypothetical molecule of zero carbon atom or may be simply understood as the functional group. Differentiating (1) with respect to $z$, one obtains $\delta_{g}^{s}G = \delta\Delta_{g}^{s}G^0/\delta z$. Thus, $\delta_{g}^{s}G$ is the change in $\Delta_{g}^{s}G^0$ per carbon atom. In addition, equation (1) can be expanded as described below [21].

From basic thermodynamics,

$$\Delta_{g}^{s}G^0 = \Delta_{g}^{s}H^0 - T\Delta_{g}^{s}S^0,$$

where $\Delta_{g}^{s}H^0$ and $\Delta_{g}^{s}S^0$ are the changes in standard enthalpy and entropy, respectively. $T$ is the absolute temperature, $k$ is the retention factor, $\beta$ is column phase ratio, and $R$ is the universal gas constant. Equation (3) is obtained by substitution of (2) into (1),

$$\ln k = -\frac{\Delta_{g}^{s}H^0}{RT} + \frac{\Delta_{g}^{s}S^0}{R} - \frac{z\delta_{g}^{s}H}{RT} + \frac{z\delta_{g}^{s}S}{R} - \ln \beta,$$

or

$$\ln \frac{t_R - t_M}{t_M} = a + bz + \frac{c}{T} + \frac{d}{T},$$

where

$$a = -\ln \beta + \frac{\Delta_{g}^{s}S^0}{R}, \quad b = \frac{\delta_{g}^{s}S}{R},$$

$$c = -\frac{\Delta_{g}^{s}H^0}{R}, \quad d = -\frac{\delta_{g}^{s}H}{R}.$$
2.3. Transesterification. Transesterification of FAMEs with other alcohols (C2–C8) was carried out via a microreactor as described by Kaewkool et al. [25]. A disposable syringe (3 mL) was plugged with a small piece of cotton wool. NaOH (0.5 g) was rapidly ground and packed into the disposable syringe.

Five hundred microliters of a standard mixture of FAMEs (C16–C20) in toluene, about 2 mg/mL each, were mixed with 0.5 mL of the desired alcohol (C2–C8). Transesterification was started by passing the mixture through the microreactor gravitationally. Elution rate was controlled manually by the plunger, such that the mixture was eluted out in 30–45 s. Another 1 mL of the toluene-alcohol (1:1 v/v) mixture was added to wash the microreactor. The wash time was about 20 s. The combined eluent was acidified with 0.1 mL glacial acetic acid and washed with 1 mL of water. The organic layer was separated and dried over anhydrous Na2SO4. The combined eluent was acidified with 0.1 mL glacial acetic acid and washed with 1 mL of water. The organic layer was separated and dried over anhydrous Na2SO4. Transesterification and soap formation were checked by a liquid chromatography-based method according to Kittiratanapiboon and Krisnangkura [26].

For coriander seed oil an equal amount of olive oil, was mixed in order to increase the oleate peak in the chromatogram. The oil was similarly transesterified as described above. For secondary octanols, alkaline catalyst was not suitable due to its slow reaction rate and soap formation. Acid catalysis was performed according to Kalayasiri et al. [27].

2.4. Gas Chromatography (GC). Gas chromatographic analysis was carried out on a Shimadzu gas chromatograph model 2010 (Shimadzu Inc., Tokyo, Japan) and equipped with an FID, a split-splitless injector, a data processor (CBM 102), and a 90%-biscyanopropyl-10%-cyanopropylphenyl polysiloxane (Rtx-2330) capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; from Restek International, Bellefonte, PA). The chromatographic conditions were as follows: Nitrogen carrier gas flow, 1 mL/min; nitrogen makeup gas flow, 30 mL/min; detector/injector, 230°C; split ratio, about 50:1. Oven temperatures were set isothermally at 5°C intervals between 180°C and 200°C, for determination of the column coefficients (a, b, c, and d) and 180°C for the separation of petroselinic and oleic esters.

2.5. Determination of the Four Coefficients of (4) for Fatty Acid Butyl Esters (FABEs). FABEs, which are approximately half way between methyl and octyl esters, were chosen as the references. The four numeric coefficients for FABEs were determined as described by Krisnangkura et al. [21].

At constant absolute temperature (T), equation (4) is reduced to (8), the well-known Martin’s equation

\[ \ln \frac{t_R - t_M}{t_M} = a' + b'z, \]  

where

\[ a' = a + \frac{c}{T}, \]

\[ b' = b + \frac{d}{T}. \]

Thus, plotting \( a' \) against \( 1/T \) would result in a straight line with a slope of \( c \) and an intercept of \( a \). Plotting \( b' \) against \( 1/T \) would also result in a straight line with a slope of \( d \) and an intercept of \( b \).

The mixture of FABEs (C16–C20) was chromatographed at 5°C intervals between 180°C and 200°C. The four column coefficients were solved by Microsoft Office Excel 2007.

2.6. Determination of the Four Coefficients of (4) for Fatty Acid Esters of Other Alcohols. The difference in retention indices of RX and RH (R = alkyl, X is functional group) called homomorphic factor. According to (1), \( \Delta G_f^G \) is the free energy contributed from the characteristic functional group, X. \( z \delta^{G_f^G} \) is the free energy contributed from the hydrocarbon chain of \( z \) carbon atoms. Thus, the difference in \( \Delta G_f^G \) between RX and RH would simply arise from the hydrocarbon chain of \( z \) carbon atoms. Similarly, two fatty acid esters having the same \( z \) but differ in the alcohol moieties, the difference in \( \Delta G_f^G \) of the two fatty acid esters would be the difference in \( \Delta \gamma \). When (1) is expanded to (4), the numeric values of \( b \) and \( d \) would remain unchanged. Only the \( a \) and \( c \) derived from \( \Delta G_f^G \), are changed. Therefore, the numeric values \( b \) and \( d \) of FABEs are assigned for other fatty acid esters. The values of \( a \) and \( c \) were obtained by solving two simultaneous equations, at different temperatures. The numeric values of \( a \) and \( c \) for each fatty acid ester are averaged from 25 data (5 different temperature pairs and 5 fatty acids).

3. Results and Discussion

3.1. The Homomorphic Factor of Fatty Acid Esters. As pointed out in Section 2.6, the homomorphic factor is the difference in retention indices of RX and RH. According to (1), two FAAEs (RICOORX) having the same R1 but differing in the alcohol moieties (R2), \( \Delta G_f^G \) would be characteristic of the homomorphic factor. In this section, attempts are made to prove that FAAEs of different alcohols would differ in \( \Delta G_f^G \), while the \( \Delta G_f^G \) values are unchanged.

3.1.1. The Numeric Values for FAAEs. The four numeric coefficients for FAAEs were determined as described in Section 2.6 and solved by Microsoft Office Excel 2007. The numeric values and the standard deviations (sd) of 95% confidence for \( a \), \( b \), \( c \), and \( d \) are listed in Table 1. Although, only 4 significant numbers are shown in Table 1, it should be pointed out that the higher decimal number would give better agreement between the predicted and experimental values. Hence, unrounded values of the 4 coefficients were used in the calculations.

The average values of \( a \) and \( c \) for FAAEs and their sd are summarized in Table 1. The values of \( a \) are more negative as the carbon numbers of the alcohols are increased. The plot of \( a \) and carbon numbers of alcohols yield a straight line with the slope of \(-0.580 \) and intercept of \(-6.265 \). The \( r^2 \), sd of intercept, and slope are 0.941, 0.298, and 0.059, respectively. On the other hand, the values of \( c \) increase positively as the carbon numbers of the alcohols are increased. The plot of
increased (Figure 1). However, the transformation of a solute increases negatively as the carbon numbers of the alcohols are
phase is measured by free energy of solution, (\Delta G_f).

The interaction between the solute and the column stationary
in solution to gas involves two physical phenomena. The
r^2, sd of intercept and slope are 0.961, 157.7, and 31.22,
respectively.

\Delta G_f^s is the difference between \Delta G_f^h and T \Delta S_f^s. The values of \Delta G_f^s for each fatty acid ester at any
temperature can be estimated from the coefficients \(a\) and \(c\) (in Table 1, with the nominal \(\beta\) value of 250). The value of \Delta G_f^s, for each fatty acid ester, at constant temperature, increases negatively as the carbon numbers of the alcohols are increased (Figure 1). However, the transformation of a solute in solution to gas involves two physical phenomena. The interaction between the solute and the column stationary phase is measured by free energy of solution, (\Delta G_f), and the vaporization of the solute is measured by free energy of vaporization, \Delta H_f^s [28, 29]. Therefore, the increment in \Delta G_f with \(R_p\) may involve \Delta G_f or \Delta H_f^s or both of them, and the separation between 18\:\Delta6cis and 18\:\Delta9cis cannot be ascribed to \Delta G_f at this moment.

3.1.2. Evaluation of Coefficients in (4). The four numeric values \((a, b, c, \text{ and } d)\) for FAAEs of different alcohols are summarized in Table 1. The validations of these values were indirectly verified by comparing the calculated retention times \((t_R^\text{cal})\) with the experimental values \((t_R^\text{exp})\). Both \(t_R^\text{cal}\) and \(t_R^\text{exp}\) values are summarized in Table 2. All the \(t_R^\text{cal}\) values are very close to the \(t_R^\text{exp}\) values. The greatest difference is 1.56% for methyl arachidate. There are only 3 \(t_R^\text{cal}\) values which differed greater than \(\pm 1.00\)% from the \(t_R^\text{exp}\) values. Forty one data points (21.0%) have the differences between 0.50 and 1.00. The rest (155 data points or 77.5%) have the differences less than 0.50%. The good agreement between the \(t_R^\text{cal}\) and \(t_R^\text{exp}\) values suggests that all the coefficients are valid for prediction retention times of FAAEs. The slopes, intercepts, \(r^2\) and standard deviations of the plots between \(t_R^\text{cal}\) and \(t_R^\text{exp}\) for each FAAE are summarized in Table 3. The \(r^2\), values of all the FAAE are greater than 0.997, and the slopes (the ratios of \(t_R^\text{cal}/t_R^\text{exp}\)) are very close to 1.00, except that of FAME.
Table 2: Comparison of the $t_R$(cal) with the $t_R$(exp) for fatty acids of different alkyl esters between temperatures 180 and 200°C, at 5°C interval.

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<tr>
<th>FAME</th>
<th>180°C $t_R$(cal)</th>
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*percent different between $t_R$(exp) and $t_R$(cal).
The report of Isbell et al. [18]. In Figure 2, the octyl esters and FAHE are baseline resolved. Results agree well with carbon atoms. FAAEs of higher alcohols (FAHpE, FAOE, acid esters are observed when the alkyl groups have 3–6 and oleic are coeluted. Partial separations of these two parentheses are standard deviation.

3. Correlation of Chromatography Research International

3.2. GC Separation of Octadecenoic Acid Alkyl Esters of Different Alcohols and Free Energy Contribution from the Double Bond. Figure 2 is the aligned chromatograms of FAAEs (with different alcohols) of a mixture of coriander seed and olive oils, separated on a 90%-cyanopropylphenyl polysiloxane (Rtx-2330) capillary column at 180°C. The methyl and ethyl esters of petroselinic and oleic are coeluted. Partial separations of these two acid esters are observed when the alkyl groups have 3–6 carbon atoms. FAAEs of higher alcohols (FAHpE, FAOE, and FAEHE) are baseline resolved. Results agree well with the report of Isbell et al. [18]. In Figure 2, the octyl esters show approximately the same resolution as those of the 2-ethyl-1-hexyl esters in separations of 18:1Δ6 and 18:1Δ9. However, the 2-ethyl-1-hexyl esters are eluted faster than the octyl esters. The retention times of the 2-ethyl-1-hexyl esters are comparable to those of the heptyl esters. The bulky 2-ethyl-1-hexyl group was ascribed for lowering the interaction between the polar carboxyl and the polar stationary phase. Therefore, the interaction between the small polarity differences of the olefin and the polar stationary phase became apparent and caused the separation [18]. Although the discussion of Isbell et al. [18] was theoretically sound, it lacked the supported evidence. On the contrary, results in Figure 1 show that Δ\text{f}G^\text{s,ln} values increase as the alkyl groups are larger, which contradicted to the explanation of Isbell et al. [18]. Therefore, a new free energy term is proposed, and it is expected that it would be able to find the cause of the separation between petroselinic and oleic esters.

Martin [20] were the first to divide a molecule into different parts, and a free energy term was assigned to each of them as shown in (1). Subsequently, different free energy terms were assigned to describe interactions between column stationary phase and different functional groups of the molecule [32, 33]. Hence, the free energy concept of Kollie and Poole [32] and Golovnya [33] was extended, in this work, to investigate the interaction between the double bond and the column stationary phase. The new free energy is called free energy of transfer from solution to gas of the double bond (Δ\text{f}G^\text{u}). Equation (10) is obtained by inserting the Δ\text{f}G^\text{u} into (1)

\[
\Delta\text{f}G^\text{u} = \frac{\Delta\text{f}G^\text{u}}{\Delta\text{f}G^\text{u}} + \frac{\Delta\text{f}G^\text{u}}{\Delta\text{f}G^\text{u}} + \frac{\Delta\text{f}G^\text{u}}{\Delta\text{f}G^\text{u}}, \tag{10}
\]

where the subscripts 1 and 2 stand for saturated and unsaturated fatty acids, respectively.

Equation (10) can also be derived as follows:

\[
\Delta\text{f}G^\text{u}_1 = -RT \ln K_1, \tag{11}
\]

\[
\Delta\text{f}G^\text{u}_2 = -RT \ln K_2. \tag{12}
\]

Equation (12) is the difference between (11).

\[
\Delta\text{f}G^\text{u}_3 = \Delta\text{f}G^\text{u}_2 - \Delta\text{f}G^\text{u}_1 = -(RT \ln K_3 - RT \ln K_1). \tag{12}
\]

Equation (10) is obtained by substituting (1) into (12).

\[
\Delta\text{f}G^\text{u}_3 = \Delta\text{f}G^\text{u}_2 - \Delta\text{f}G^\text{u}_1 = \Delta\text{f}G^\text{u}_3 = \Delta\text{f}G^\text{u}_3 - \Delta\text{f}G^\text{u}_3 \tag{13}
\]

The Δ\text{f}G^\text{u}_3 will be used as a tool to investigate the interaction of the double bond with the stationary phase of the column. The Δ\text{f}G^\text{u}_3 values of petroselinic and oleic (and vaccenic 18:1Δ11cis) acid esters at 180°C are summarized in Table 4. The C1–C3 esters are not well separated from each other, and they are not listed in the table. The Δ\text{f}G^\text{u}_3 values of these three esters at 180°C are approximately −151, −133, and 128 cal·mol⁻¹, respectively. Partial separations are observed for FABEs. Separations of FAAE are gradually improved as the carbon numbers of the alcohols are increased.

According to Isbell et al. [18], the bulky alkyl group interfered the interaction of the carboxyl group to the column stationary phase. The interference did not extend to the hydrocarbon chain. Hence, the relative changes in Δ\text{f}G^\text{s} should be the same for both saturated and unsaturated FAAEs. However, the Δ\text{f}G^\text{s} values of petroselinic, oleic, and vaccenic esters (Table 4) become less negative as the alkyl sizes are increased, suggesting that the alkyl groups interfere the interaction between the hydrocarbon chain and the stationary phase, and the degree of interferences increases as the carbon numbers of the alcohols are increased.

The Δ\text{f}G^\text{s} values of the butyl esters of 18:1Δ6, Δ9, and Δ11 are −102.4, −120.1, and −145.4 cal·mol⁻¹, respectively. The values become less negative as the alkyl groups are larger. 2-Ethyl-1-hexyl esters have the lowest Δ\text{f}G^\text{s} compared with other alkyl esters. Their Δ\text{f}G^\text{s} values for 18:1Δ6, Δ9, and Δ11 are −36.7, −61.4, and −90.5 cal·mol⁻¹, respectively.

Table 3: Correlation of \( t_{R(id)} \) and \( t_{R(exp)} \) of FAAEs. Numbers in parentheses are standard deviation.

<table>
<thead>
<tr>
<th>FAAE</th>
<th>Slope</th>
<th>Intercept</th>
<th>( r^2 )</th>
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<tbody>
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<td>FAME</td>
<td>0.970 (0.004)</td>
<td>0.162 (0.021)</td>
<td>0.997</td>
</tr>
<tr>
<td>FAE</td>
<td>0.987 (0.002)</td>
<td>0.086 (0.015)</td>
<td>0.999</td>
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<tr>
<td>FAPrE</td>
<td>0.995 (0.002)</td>
<td>0.034 (0.013)</td>
<td>0.999</td>
</tr>
<tr>
<td>FABE</td>
<td>1.001 (0.003)</td>
<td>0.025 (0.023)</td>
<td>0.999</td>
</tr>
<tr>
<td>FAPnE</td>
<td>0.999 (0.002)</td>
<td>0.004 (0.025)</td>
<td>0.999</td>
</tr>
<tr>
<td>FAHxE</td>
<td>1.003 (0.002)</td>
<td>−0.044 (0.021)</td>
<td>0.999</td>
</tr>
<tr>
<td>FAHpE</td>
<td>1.008 (0.001)</td>
<td>−0.112 (0.021)</td>
<td>0.999</td>
</tr>
<tr>
<td>FAOE</td>
<td>1.007 (0.002)</td>
<td>−0.121 (0.038)</td>
<td>0.999</td>
</tr>
</tbody>
</table>
Therefore, the interaction of the double bond with the column stationary phase increases when the double bond is far away from the carboxyl group, that is, interference from the bulky alkyl group decreases along the distance.

The difference in ΔΔ\textsubscript{ln} G\textsubscript{u} of 18:1Δ9 and 18:1Δ6 (ΔΔ\textsubscript{ln} G\textsubscript{u}(Δ9−Δ6)) of butyl, pentyl, hexyl, heptyl, octyl and 2-ethylhexyl esters is −17.7, −21.8, −23.5, −23.3, −24.5, and −24.7 cal·mol\textsuperscript{−1}, respectively. The increases in ΔΔ\textsubscript{ln} G\textsubscript{u}(Δ9−Δ6) as the alkyl groups increased suggest that the relative interactions of the double bonds of the two acid esters with the stationary phase are increased. Thus, it may conclude that ΔΔ\textsubscript{ln} G\textsubscript{u}(Δ9−Δ6) is the driving force for the separation of the two isomers. However, the ΔΔ\textsubscript{ln} G\textsubscript{u}(Δ9−Δ6) values level after C6, implying that the resolution of these two positional isomers may not be further increased by simply increasing the size of the alcohol.

The ΔΔ\textsubscript{ln} G\textsubscript{u}(Δ9−Δ6) of 2-ethyl-1-hexyl esters is −24.7 cal·mol\textsuperscript{−1}. It is estimated that a column of about 65,000 required plate numbers (N\textsubscript{req}) would be able to baseline separate the two positional isomers, while the butyl esters, whose ΔΔ\textsubscript{ln} G\textsubscript{u}(Δ9−Δ6) is −17.7 cal·mol\textsuperscript{−1}, would require up to 180,000 plate numbers. The ΔΔ\textsubscript{ln} G\textsubscript{u} of 18:1Δ9 and Δ11 have similar trend as the 18:1Δ6 and 18:1Δ9 pair, and discussion is not necessary.

Petroselinic acid has a double bond closer to the carboxyl group than oleic acid. The interference from the bulky alkyl group should be stronger than that to the oleic acid. Similar observation was reported by Kuningas et al. [34] that the separation of geometric isomers (alkenes) depended on the differences in free energy.

The ΔΔ\textsubscript{ln} G\textsubscript{u}(Δ9−Δ6) the adjacent pairs of octadecenoic esters would reflect on the ease of separation for each pair. Results in Table 4 show that the separation of 18:1Δ9 and 18:1Δ11 is much simpler than the separation of 18:1Δ6 and 18:1Δ9.

3.3. Separation of Octadecenoic Acid Octyl Esters. As discussed in Section 3.2, the lowering in ΔΔ\textsubscript{ln} G\textsubscript{u} was ascribed to the reduction in the interaction between the double bond in the FAAEs and the column stationary phase. 1-Octyl and 2-ethyl-1-hexyl esters are two of the best derivatives for separation of petroselinic and oleic acids. However, esters of secondary octyl alcohols have not been reported (to our knowledge). Thus, they were investigated for their interferences to the interaction of the double bond with the stationary phase. The retention times, retention factors, ΔΔ\textsubscript{ln} G\textsubscript{u}(Δ9−Δ6), and resolution (Rs) between secondary octyl petroselinic and oleate are summarized in Table 5. The ΔΔ\textsubscript{ln} G\textsubscript{u}(Δ9−Δ6) values of secondary octyl esters of petroselinic and oleate range from −24.5 to −25.8 cal·mol\textsuperscript{−1}. The resolutions of these two acid secondary octyl esters (1.15-1.26) are close to that of the 1-octyl ester (1.26). However, more symmetric or compact alcohol had the shorter retention time. The order of elution for the esters is 4-octyl, 3-octyl, 2-octyl, and 1-octyl, respectively.

4. Conclusion

When the alcohol moieties of FAAE are changed, the ΔΔ\textsubscript{ln} G\textsubscript{u} does not change. It implies that the interaction between the saturated hydrocarbon chain and the stationary phase is not affected by the alcohol moieties. On the other hand, petroselinic and oleic have one cis-double bond in the hydrocarbon chain; its interaction with the column stationary phase would differ from that of the saturated hydrocarbon. Therefore, ΔΔ\textsubscript{ln} G\textsubscript{u} is introduced and it would provide an insight into the interaction of the double with the column stationary phase. The bulky alkyl group attached to the carboxyl group affects the interaction between the double bond and the stationary phase. The steric effect decreases along the distance. In addition, for FAAEs having the same

### Table 4: The ΔΔ\textsubscript{ln} G\textsubscript{u}(cal·mol\textsuperscript{−1}) of esters of C18:1Δ6, C18:1Δ9, and C18:1Δ11.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>FAEE</th>
<th>FAPnE</th>
<th>FAExE</th>
<th>FAHpE</th>
<th>FAOE</th>
<th>FAEHE</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>18:1Δ6</td>
<td>−102.4</td>
<td>−82.0</td>
<td>−78.7</td>
<td>−68.8</td>
<td>−61.4</td>
<td>−36.7</td>
</tr>
<tr>
<td>18:1Δ9</td>
<td>−120.1</td>
<td>−103.8</td>
<td>−102.2</td>
<td>−92.1</td>
<td>−85.9</td>
<td>−61.4</td>
</tr>
<tr>
<td>18:1Δ11</td>
<td>−145.4</td>
<td>−134.3</td>
<td>−131.7</td>
<td>−122.1</td>
<td>−113.1</td>
<td>−90.5</td>
</tr>
</tbody>
</table>

### Table 5: Separations of petroselinic and oleic octyl esters on Rtx 2330 at 190°C.

<table>
<thead>
<tr>
<th>Octanol</th>
<th>t\textsubscript{E1}(min)\textsuperscript{a}</th>
<th>t\textsubscript{E2}(min)</th>
<th>k\textsubscript{1}</th>
<th>k\textsubscript{2}</th>
<th>ΔΔ\textsubscript{ln} G\textsubscript{u}(Δ9−Δ6) (cal·mol\textsuperscript{−1})</th>
<th>Rs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-octanol</td>
<td>17.27</td>
<td>17.71</td>
<td>6.19</td>
<td>6.35</td>
<td>−24.5</td>
<td>1.26</td>
</tr>
<tr>
<td>2-octanol</td>
<td>12.67</td>
<td>12.97</td>
<td>4.51</td>
<td>4.64</td>
<td>−25.8</td>
<td>1.15</td>
</tr>
<tr>
<td>3-octanol</td>
<td>12.00</td>
<td>12.29</td>
<td>4.00</td>
<td>4.12</td>
<td>−25.1</td>
<td>1.17</td>
</tr>
<tr>
<td>4-octanol</td>
<td>11.41</td>
<td>11.69</td>
<td>3.74</td>
<td>3.84</td>
<td>−25.1</td>
<td>1.20</td>
</tr>
</tbody>
</table>

\textsuperscript{a}the subscripts 1 and 2 represent petroselinate and oleate, respectively.
carbon numbers, the more compact is alcohol moiety, the shorter is the retention time, while the resolutions of the adjacent positional isomers are approximately the same. For petroselinic and oleic acids, the driving force for their separation is the difference in $\Delta f_{1n} G_n$ of the two geometrical isomers.

5. Symbols and Abbreviation

Fatty Acids

18:1Γ6cis: Petroselinic acid
18:1Γ9cis: Oleic acid
18:1Γ11cis: Vacenic acid.

Thermodynamics

$\Delta f_{1n} G^o$: Standard free energy of transfer from solution to gas
$\Delta f_{1n} H^o$: Standard enthalpy of transfer from solution to gas
$\Delta f_{1n} S^o$: Standard entropy of transfer from solution to gas
$\Delta f_{1n} G_f$: Free energy of transfer from solution to gas of a hypothetical molecule of zero carbon atom or simply called free energy of the functional gr
$\Delta f_{1n} H_f$: Enthalpy of transfer from solution to gas of a hypothetical molecule of zero carbon atom
$\Delta f_{1n} S_f$: Entropy of transfer from solution to gas of a hypothetical molecule of zero carbon atom
$\Delta f_{1n} G_u$: Free energy of transfer from solution to gas of a double bond
$\delta f_{1n} G$: Change in free energy of transfer from solution to gas per carbon atom
$\delta f_{1n} H$: Change in enthalpy of transfer from solution to gas per carbon atom
$\delta f_{1n} S$: Change in entropy of transfer from solution to gas per carbon atom

$\Delta f_{1n} G_{a(\Delta 9-\Delta 6)}$: The difference in free energy of transfer from solution to gas of two unsaturated FFAE (Δ9 and Δ6).

General

$\beta$: Column phase ratio
FFAE: Fatty acid alkyl ester
FABE: Fatty acid butyl ester
FAEE: Fatty acid ethyl ester
FAEHE: Fatty acid 2-ethylhexyl ester
FAHPe: Fatty acid heptyl ester
FAHxe: Fatty acid hexyl ester
FAME: Fatty acid methyl ester
FApNe: Fatty acid pentyl ester
FAPHe: Fatty acid propyl ester
FAOe: Fatty acid octyl ester

k: Retention factor
R: Universal gas constant
Rs: Resolution
sd: Standard deviation
$t_f$: Retention time of a solute
$t_M$: Hold up time
T: Absolute temperature
z: Carbon number.

Acknowledgment

This work was supported by Thailand Research Fund.

References

[13] F. Santinelli, P. Damiani, and F. Vandamme, "Separation of petroselinic (cis-6 18:1) and oleic (cis-9 18:1) acids by gas-liquid...


