

Prediction of Gas Chromatographic Retention Times of Esters of Long Chain Alcohols and Fatty Acids

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Abstract

The linear free energy of solution (ΔG) relationship ($\Delta G = \Delta G_o + z\delta G$) for compounds of different carbon atoms (z) in the same homologous series is expanded and modified to cover compounds with two different hydrocarbon side chains. The expanded equation is successfully used to predict the retention times (t_R) of standard esters of long chain alcohols and fatty acids of different chain lengths in both isothermal and temperature-programmed gas chromatography (TPGC). Approximately 90% of the 125 predicted t_R values have a difference of less than 1.00% from the actual t_R and the highest difference is 1.26%. Two different temperature gradients in TPGC are tested. The expanded equation can be used to forecast the t_R of TPGC with good accuracy. The highest difference is $\pm 1.40\%$ and $\pm 1.00\%$ for the temperature gradients of 2°C and 4°C/min, respectively. However, the increments in free energy per carbon atom ($z\delta G$) of the alcohol and acid are approximately equal but have slightly different temperature sensitivities. Therefore, it is very difficult to separate esters of different acid and alcohol chain length but with the same total carbon numbers. Furthermore, the difference in temperature sensitivities for the acid and alcohol side chains renders them to be inversely eluted at different temperatures.

Introduction

Kovats' retention index system has been used extensively as an aid in gas chromatographic (GC) identification of organic compounds (1–3). Using *n*-alkanes as the references has a drawback, especially for the identification of high molecular weight and polar compounds, which requires high molecular *n*-alkanes (4). Thus, other similar systems, which use different standard references compounds [e.g., equivalent chain length, carbon number (5,6), or ketone number (7)] have been set up. Waxes are ubiquitous in nature, but there is no identification system for them. Furthermore, retention indices are not available. With the price of GC–mass spectrometers (MS) low enough now that many laboratories can afford them, waxes are then identified by their mass

spectra (8–10). Otherwise, they are usually identified by comparison of their retention times (t_R) with reference waxes, which are difficult to obtain in chemically pure forms. Therefore, tentative identification of a wax, for those without access to an MS, requires standard wax as the reference. However, beeswax may be hydrolyzed to long chain alcohols and acids (9), which are easier to identify, and the relatively expensive reference waxes are avoided, but the identity of the wax is lost.

With recent advances in knowledge of solute migration along the GC column, the t_R of several solutes can be forecast with good accuracy, but there is no report on the prediction of t_R of waxes. In this study, a thermodynamic model is proposed to predict the t_R of waxes of different alcohols and fatty acids. The basic equation proposed by Krisnangkura et al. (equation 1) (11), which is used to predict the t_R of fatty acid methyl esters (FAMES) of fixed alcohol chain length, is expanded to cover alcohols of variable chain lengths.

$$\ln k = a + bz + \frac{c}{T} + \frac{dz}{T} \quad \text{Eq. 1}$$

where a , b , c , and d are thermodynamically related column constants.

The well-known free energy of solution (ΔG) equation of James and Martin, equation 2, is further expanded to equation 4.

$$\Delta G = \Delta G_o + z_i \delta G_i \quad \text{Eq. 2}$$

where z_i is the carbon number of fatty acid. ΔG_o is the free energy of a solution of hypothetical acid of a zero carbon atom (with a fixed number of carbon atoms of the alcohol). δG_i is the increment in free energy of solution per carbon atom of the acid. When the alcohols vary, James and Martin's equation is repeated as shown in equation 3.

$$\Delta G_o = \Delta G_{oo} + z_j \delta G_j \quad \text{Eq. 3}$$

where z_j is the carbon number of alcohol, and ΔG_{oo} is the free energy of a solution of hypothetical ester of a zero carbon atom of both alcohol and acid. δG_j is the increment in free energy of solution per carbon atom of the alcohol.

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If there are no interactions between the two side chains of the acid and alcohol, equations 2 and 3 are directly combined.

$$\Delta G = \Delta G_{oo} + z_i \delta G_i + z_j \delta G_j \quad \text{Eq. 4}$$

From basic thermodynamics, the free energy of solutions in equation 4 can be expanded to:

$$\ln \frac{t_R - t_M}{t_M} = -\ln \beta - \frac{\Delta H_{oo}}{RT} + \frac{\Delta S_{oo}}{R} - \frac{z_i \delta H_i}{RT} + \frac{z_i \delta S_i}{R} - \frac{z_j \delta H_j}{RT} + \frac{z_j \delta S_j}{R} \quad \text{Eq. 5}$$

or

$$\ln k = a + b_i z_i + b_j z_j + \frac{c}{T} + \frac{d_i z_i}{T} + \frac{d_j z_j}{T} \quad \text{Eq. 6}$$

where

$$a = -\ln \beta + \frac{\Delta S_{oo}}{R} \quad \text{Eq. 7}$$

$$b = \frac{\delta S}{R} \quad \text{Eq. 8}$$

$$c = -\frac{\Delta H}{R} \quad \text{Eq. 9}$$

$$d = -\frac{\delta H}{R} \quad \text{Eq. 10}$$

Thus, equation 6 is proposed for prediction of t_R of waxes of variable chain lengths in the acid and alcohol moieties.

Experimental

Fatty acids (14–18 carbon atoms) and long chain alcohols (14–22 carbon atoms) were purchased from Sigma Chemical Co. (St. Louis, MO). Natural beeswax was obtained from a grocery store (Bangkok, Thailand). Esterification of long chain fatty acid and long chain alcohol was modified from Kalayasiri et al. (12) for methyl ester with 2% H_2SO_4 as the catalyst, but the reaction time for esterification of long chain alcohols and acid was extended to 24 h at 80°C.

GC

GC analysis was performed on a Shimadzu (Kyoto, Japan) model 17A. The instrument was equipped with a flame ionization detector and split/splitless injector. Separation of the esters was performed on an AT-5 high-temperature capillary column (10-m \times 0.1-mm i.d., 0.1- μm film thickness) from Alltech Assoc. (Deerfield, IL). Nitrogen was used as carrier gas at the flow rate of 0.18–0.2 mL/min. Injector and detector temperatures were set at 350°C. The column oven was held isothermally at intervals between 290°C and 310°C. Temperature programming conditions are reported in the text.

Determination of the constants of equation 6

Determination of the six numeric constants of equation 6 can be done by holding each variable constant at a time.

When the fatty acid carbon number (z_i) is fixed, equation 6 is reduced to:

$$\ln k = a' + b_j z_j + \frac{b'}{T} + \frac{d_j z_j}{T} \quad \text{Eq. 11}$$

where

$$a' = a + b_i z_i \quad \text{Eq. 12}$$

$$b' = c + d_i z_i \quad \text{Eq. 13}$$

Equation 11 is similar to that proposed by Krisnagkura et al. (11). Thus the four numeric constants of equation 11 can be solved as described. Combining terms (with constant z_j), equation 11 is further reduced to:

$$\ln k = a'' + \frac{b''}{T} \quad \text{Eq. 14}$$

where

$$a'' = a' + b_j z_j \quad \text{Eq. 15}$$

and

$$b'' = b' + d_j z_j \quad \text{Eq. 16}$$

The plots of $\ln k$ versus $1/T$, at different z_j , yield different intercepts (a'') and different slopes (b''). Each z_j would give an intercept of a'' and slope of b'' . Thus, the plot of a'' versus z_j , according to equation 15 would give an intercept of a' and slope of b_j . Similarly, the plot between b'' and z_j , according to equation 16, would give an intercept of b' and slope of d_j .

The numeric constants (a and b_i and c and d_i) are obtained from the intercepts and slopes of the a'' - z_j and b'' - z_j plots (as described in equations 12 and 13, respectively).

Results and Discussion

Adjustment of the predicting equation

The natural logarithm of retention factors for esters of long chain alcohols and fixed fatty acids on the AT-5 column are summarized in Table I, and the numeric values of the four constants of equation 6 are included. By comparing the four numeric constants in equation 6 to those obtained from methyl esters on a nonpolar 100% dimethyl polysiloxane (OV-101, 15-m \times 0.25-mm i.d.) (13), it was obvious that the increments in enthalpy and entropy per carbon atom were very close, and the standard enthalpy and entropy of solution for hypothetical zero carbon atom were much larger than those of the methyl esters. The differences were wider as the fatty acid chain length is increased. These differences arose from the differences in the number of carbon atoms of the hypothetical zero carbon atom of the wax, which still have 14–18 carbon atoms of the acids left in the

molecules, and in the methyl ester, there was only one carbon, of the methyl group, left in the molecule. The entropic constant, a' , becomes more negative as the carbon atoms of the acids increase. On the contrary, the enthalpic term, b' , increases as the carbon atoms of the acids are increased. Both a' and b' vary linearly with carbon numbers of the acids, and the correlation coefficients are 0.980 and 0.990, respectively, (Table II).

The a' - z_i plot (Table II), according to equation 12, gave the intercept (a) at -3.82 with the slope (b_i) of -0.51. Similarly, the b' - z_i plots, according to equation 13, gave the intercept (c) and the slope (d_i) of -805.92 and 416.58, respectively. It was mentioned earlier that equation 4 was derived on the assumption there were no interactions between the two side chains of fatty acid and alcohol. Alternatively, the fatty acid side chain should not affect or change the numerical constants, b_j and d_j or vice versa. However, the results in Table II show that the numeric values, both b_j and d_j , change linearly with the fatty acid chain length. The correlation coefficients are 0.9896 and 0.9909, respectively. Thus, experimental results in Table II suggest that the acid side chain had a

certain influence on the numeric value of the constants b_j and d_j . By adding the two linear regressions into equation 6, a modified equation 17 is obtained:

$$\ln k = a + b_i z_i + (e + f z_i) z_j + \frac{c}{T} + \frac{d_i z_i}{T} + \frac{(g + h z_i) z_j}{T} \quad \text{Eq. 17}$$

By substituting all the numeric values in Table II into equation 17, equation 18 is obtained:

$$\ln k = -3.817 - 0.509 z_i - (0.615 - 0.015 z_i) z_j - \frac{805.92}{T} + \frac{416.58 z_i}{T} + \frac{(476.66 - 9.03 z_i) z_j}{T} \quad \text{Eq. 18}$$

or

$$\ln k = -3.817 - 0.509 z_i - 0.615 z_j - 0.015 z_i z_j - \frac{805.92}{T} + \frac{416.58 z_i}{T} + \frac{476.66 z_j}{T} - \frac{9.03 z_i z_j}{T} \quad \text{Eq. 19}$$

Table I. Natural Logarithm of Retention Factors of Esters of Long Chain Alcohols and Fatty Acids at Different Temperature*

z_i	Temperature					Intercept (a')	Slope (b')	r	Intercept (a' - z_i plot)	Slope (b' - z_i plot)
	290°C	295°C	300°C	305°C	310°C					
($z_i = 14$)										
14	1.027	0.852	0.710	0.561	0.422	-16.49	9862.08	0.9994		
16	1.464	1.281	1.127	0.965	0.814	-17.40	10616.86	0.9996		
18	1.900	1.706	1.540	1.370	1.206	-18.22	11326.19	0.9997		
20	2.331	2.129	1.951	1.772	1.596	-18.99	12002.39	0.9998	-10.92 (a')	5011.77 (b')
22	2.761	2.549	2.362	2.172	1.986	-19.72	12659.06	0.9999	-0.403 (b_j)	348.98 (d_j)
($z_i = 15$)										
14	1.244	1.063	0.909	0.768	0.621	-16.75	10125.71	0.9992		
16	1.679	1.487	1.322	1.171	1.011	-17.61	10854.58	0.9993		
18	2.112	1.910	1.735	1.572	1.402	-18.41	11550.65	0.9995		
20	2.541	2.329	2.145	1.971	1.791	-19.15	12207.32	0.9996	-11.33 (a')	5373.71 (b')
22	2.969	2.747	2.552	2.369	2.179	-19.88	12864.22	0.9996	-0.391 (b_j)	341.49 (d_j)
($z_i = 16$)										
14	1.466	1.277	1.115	0.966	0.815	-17.37	10599.06	0.9992		
16	1.900	1.694	1.526	1.368	1.205	-18.14	11275.95	0.9990		
18	2.332	2.114	1.935	1.770	1.595	-18.90	11946.11	0.9999		
20	2.759	2.533	2.343	2.168	1.983	-19.62	12596.15	0.9992	-12.18 (a')	5989.16 (b')
22	3.186	2.947	2.748	2.564	2.370	-20.34	13240.26	0.9991	-0.372 (b_j)	330.13 (d_j)
($z_i = 17$)										
14	1.685	1.487	1.324	1.170	1.010	-17.78	10953.50	0.9992		
16	2.119	1.910	1.737	1.572	1.406	-18.48	11591.09	0.9992		
18	2.546	2.329	2.145	1.969	1.794	-19.22	12247.76	0.9994		
20	2.973	2.746	2.552	2.366	2.181	-19.95	12904.54	0.9994	-12.66 (a')	6385.86 (b')
22	3.398	3.161	2.956	2.762	2.566	-20.68	13554.82	0.9995	-0.364 (b_j)	325.80 (d_j)
($z_i = 18$)										
14	1.885	1.705	1.536	1.379	1.217	-17.51	10918.52	0.9999		
16	2.315	2.127	1.948	1.780	1.607	-18.25	11581.47	0.9999		
18	2.742	2.544	2.354	2.180	1.990	-19.05	12270.59	0.9999		
20	3.166	2.959	2.760	2.574	2.385	-19.55	12790.41	0.9999	-12.80 (a')	6588.61 (b')
22	3.590	3.368	3.162	2.968	2.768	-20.26	13430.55	0.9999	-0.340 (b_j)	311.65 (d_j)

* The intercepts (a'), slopes (b'), and correlation coefficient of the $\ln k$ - z_i plot are included. The last two columns are the intercepts and slopes of the a' - z_i and b' - z_i plots of different z_i .

Table II. All Eight Numeric Values of Equation 18 are Obtained from the Intercepts and Slope of the $a'-z_1$, $c-z_1$, $b'-z_1$, and $d-z_1$ Plots

z_1	a'	b'_j	b'	d'_j
14	-10.92	-0.40	5011.77	348.98
15	-11.33	-0.39	5373.71	341.49
16	-12.18	-0.37	5989.16	330.13
17	-12.66	-0.36	6385.86	325.80
18	-12.80	-0.34	6588.61	311.65
Intercept	-3.817 (<i>a</i>)	-0.615 (<i>e</i>)	-805.92 (<i>c</i>)	476.66 (<i>g</i>)
Slope	-0.509 (<i>b</i>)	0.015 (<i>f</i>)	416.58 (<i>d</i>)	-9.03 (<i>h</i>)
Correlation coefficient	-0.9806	0.9896	0.9904	-0.9909

Equation 19 is then used to predict the t_R of the esters of long chain fatty acids and alcohols.

Prediction of t_R of standard esters of long chain fatty acids and alcohols

Table III shows the percent differences (% Δ) between the experimental and predicted t_R of esters of long chain alcohols and fatty acids of different chain lengths at temperatures between 290°C and 310°C. All t_R predicted were very close to the experimental values. Seventy-three data points (58.4%) had percent differences between the experimental and predicted t_R of less than 0.50%, and only 12 data points (9.6%) had differences greater than 1.00%. The highest difference was 1.26%.

The increment in entropy per carbon atoms for the alcohol

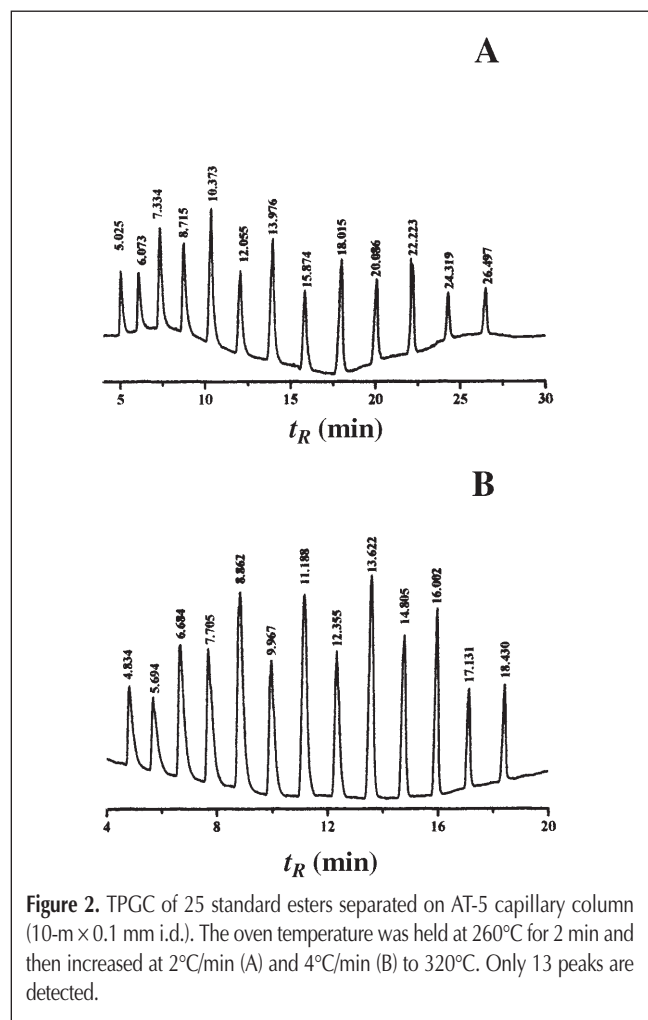
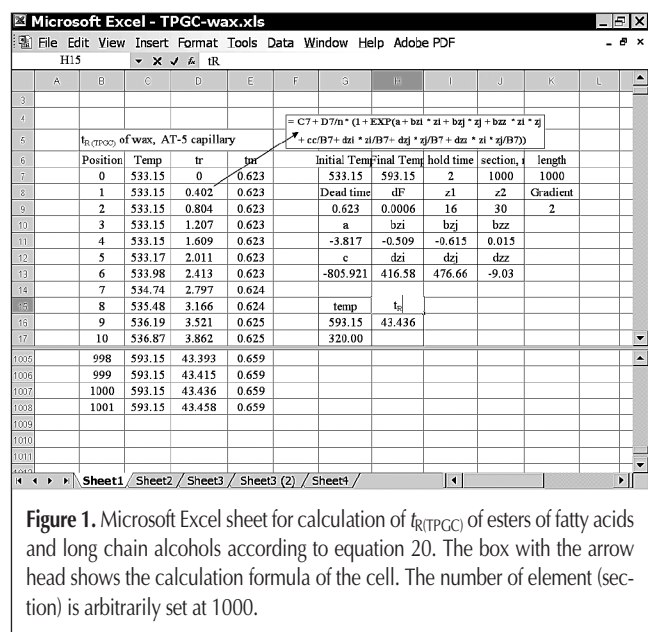
Table III. Comparison of Experimental ($t_{R(\text{exp})}$) and Forecasted t_R ($t_{R(\text{cal})}$) (min) Using Equation 19 of Fatty Acid Esters of Long Chain Alcohols at Temperatures 290–310°C

	290°C		295°C		300°C		305°C		310°C	
	$t_{R(\text{exp})}^*$	$t_{R(\text{cal})}^\dagger$ (% Δ)	$t_{R(\text{exp})}$	$t_{R(\text{cal})}$ (% Δ)	$t_{R(\text{exp})}$	$t_{R(\text{cal})}$ (% Δ)	$t_{R(\text{exp})}$	$t_{R(\text{cal})}$ (% Δ)	$t_{R(\text{exp})}$	$t_{R(\text{cal})}$ (% Δ)
$z_1 = 14$										
14	2.44	2.43 (0.58)	2.19	2.21 (-0.82)	2.00	1.99 (0.05)	1.81	1.81 (-0.20)	1.67	1.66 (0.47)
16	3.43	3.40 (0.89)	3.02	3.03 (-0.45)	2.69	2.68 (0.41)	2.38	2.38 (0.03)	2.15	2.14 (0.62)
18	4.95	4.89 (1.17)	4.27	4.28 (-0.26)	3.73	3.71 (0.56)	3.24	3.23 (0.29)	2.87	2.85 (0.75)
20	7.27	7.20 (1.03)	6.17	6.19 (-0.29)	5.29	5.26 (0.51)	4.52	4.50 (0.34)	3.92	3.89 (0.72)
22	10.83	10.75 (0.73)	9.05	9.10 (-0.55)	7.64	7.61 (0.37)	6.42	6.40 (0.24)	5.48	5.44 (0.63)
t_M	0.644		0.656		0.657		0.658		0.661	
$z_1 = 15$										
14	2.88	2.86 (0.53)	2.56	2.58 (-0.62)	2.29	2.30 (-0.22)	2.07	2.07 (0.10)	1.89	1.88 (0.39)
16	4.10	4.05 (0.88)	3.56	3.59 (-0.46)	3.13	3.14 (-0.06)	2.78	2.77 (0.31)	2.48	2.46 (0.46)
18	5.97	5.89 (1.07)	5.09	5.12 (-0.29)	4.39	4.40 (0.080)	3.82	3.80 (0.42)	3.35	3.32 (0.56)
20	8.82	8.73 (0.90)	7.39	7.45 (-0.52)	6.28	6.30 (-0.02)	5.37	5.35 (0.37)	4.62	4.59 (0.54)
22	13.19	13.09 (0.56)	10.89	11.01 (-0.82)	9.10	9.16 (-0.30)	7.68	7.66 (0.22)	6.50	6.47 (0.36)
t_M	0.645		0.654		0.655		0.658		0.662	
$z_1 = 16$										
14	3.42	3.41 (0.42)	3.01	3.02 (-0.53)	2.67	2.68 (-0.53)	2.39	2.39 (-0.07)	2.15	2.15 (0.26)
16	4.93	4.89 (0.88)	4.24	4.26 (-0.38)	3.69	3.71 (-0.44)	3.24	3.24 (0.15)	2.86	2.85 (0.38)
18	7.25	7.17 (1.15)	6.11	6.13 (-0.38)	5.22	5.25 (-0.47)	4.52	4.50 (0.43)	3.91	3.89 (0.44)
20	10.78	10.67 (1.03)	8.94	8.98 (-0.50)	7.52	7.56 (-0.60)	6.41	6.39 (0.36)	5.46	5.43 (0.40)
22	16.17	16.04 (0.80)	13.19	13.32 (-0.98)	10.95	11.05 (-0.95)	9.21	9.19 (0.14)	7.72	7.70 (0.17)
t_M	0.647		0.654		0.657		0.658		0.660	
$z_1 = 17$										
14	4.10	4.08 (0.54)	3.57	3.59 (-0.62)	3.14	3.15 (-0.40)	2.78	2.78 (0.04)	2.48	2.47 (0.41)
16	5.99	5.91 (1.20)	5.10	5.10 (0.11)	4.40	4.40 (0.06)	3.83	3.81 (0.33)	3.35	3.33 (0.63)
18	8.83	8.72 (1.26)	7.41	7.40 (0.16)	6.29	6.28 (0.06)	5.37	5.35 (0.31)	4.63	4.60 (0.64)
20	13.19	13.03 (1.26)	10.91	10.89 (0.19)	9.12	9.12 (0.04)	7.67	7.65 (0.27)	6.51	6.46 (0.62)
22	19.85	19.63 (1.11)	16.18	16.20 (-0.11)	13.33	13.37 (-0.32)	11.08	11.06 (0.13)	9.25	9.22 (0.38)
t_M	0.648		0.656		0.657		0.658		0.660	
$z_1 = 18$										
14	4.97	4.96 (0.07)	4.27	4.29 (-0.42)	3.72	3.73 (-0.31)	3.27	3.25 (0.53)	2.89	2.87 (0.38)
16	7.29	7.25 (0.58)	6.17	6.16 (0.12)	5.28	5.27 (0.19)	4.56	4.52 (0.92)	3.95	3.92 (0.63)
18	10.82	10.74 (0.69)	9.02	9.00 (0.20)	7.60	7.58 (0.20)	6.48	6.40 (1.26)	5.48	5.46 (0.38)
20	16.19	16.09 (0.59)	13.32	13.30 (0.15)	11.07	11.05 (0.17)	9.29	9.19 (1.13)	7.82	7.73 (1.12)
22	24.30	24.28 (0.09)	19.73	19.81 (-0.45)	16.22	16.25 (-0.21)	13.46	13.34 (0.91)	11.16	11.07 (0.74)
t_M	0.655		0.657		0.659		0.658		0.661	

* $t_{R(\text{exp})}$ is the retention obtained from the GC.

† $t_{R(\text{cal})}$ is the forecasted or calculated t_R .

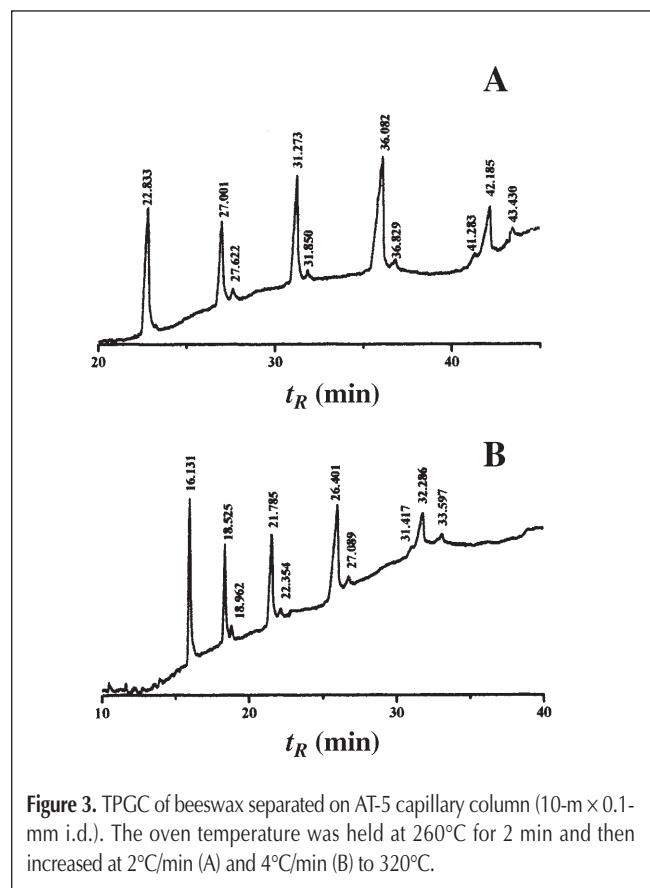
(-0.615) was more negative than that of the acid (-0.509). On the other hand, the increment in enthalpy per carbon atom for the alcohol (476.66) was more positive than the acid (416.58). When the enthalpies were divided by the absolute temperature, 563.15



K-568.15 K (290–295°C), the increments in enthalpy and entropy per carbon atom are almost nullified. Therefore, it is speculated that in this temperature range, the esters, which have the same carbon numbers, should have approximately the same k value, and they should not be separated or poorly separated. This was confirmed by the experimental data in Table I. Furthermore, the order of elution of the esters having the same total carbon numbers cannot be generalized. It depended on temperature and the chain length of the acid and the alcohol. Inversion of elution was found in almost all cases at the temperature range of 290–310°C. For example, for esters of 30 carbon atoms, the acid of 14 carbon atoms and alcohol of 16 carbon atoms (14/16 ester) had a larger k , at 290°C, than 16/14 ester, but inversion was observed at temperature \geq 295°C. For esters of 32 carbon atoms, the 14/18 ester had a larger k than the 16/16 ester at 290–295°C, and an inversion was found at 300°C, and the 18/14 ester had the larger k at all temperatures.

Prediction of retention times of standard esters of long chain fatty acids and alcohols in TPGC

The column slicing method of Calvalli and Guinchard (14) was an excellent technique for determination of t_R in TPGC. The column was arbitrarily divided into very short columns of the predetermined elements. The t_R of TPGC [$t_{R(TPGC)}$] of a solute was the sum of the t_R of each element, whose temperature was determined by the time the solute entered the element. Although Calvalli and Guinchard (14) used the method for predicting an individual solute, Kittiratanapiboon et al. (15) extended and slightly modified the method to predict the $t_{R(TPGC)}$ of compounds



of the same homologous series from their carbon numbers or retention indices. In this study, equation 19 was modified according to Kittiratanapiboon et al. (15) to equation 20 and used to predict the $t_{R(\text{TPGC})}$ of esters of long chain alcohols and fatty acids.

$$t_R = \sum_{i=1}^m \frac{t_M [1 + g(\theta_i - T_i)]}{m} \left[1 + e \left(-3.817 - 0.509z_i - 0.615z_j + 0.015z_i z_j - \frac{805.92 - 416.58z_i - 476.66z_j + 9.03z_i z_j}{T} \right) \right] \quad \text{Eq. 20}$$

where m is the number of elements (column being sliced, = 1000); t_M is the gas hold-up time at the initial temperature (T_i); q_i is the absolute temperature of the i th element; g is the hold-up time adjustment factor because of the change in temperature. The MS Excel (2003) (Microsoft, Redmond, WA) calculation sheet was slightly modified as shown in Figure 1. The exponents of the calculation formula in column D (in Figure 1) were changed to those described in equation 20, in which all of the numeric constants were given in the frame on the right hand side.

Figure 2 is the gas chromatograms of standard esters chromatographed at 260°C to 320°C at a temperature gradient of

2°C and 4°C/min, respectively. The initial hold times were 2 min for both chromatograms. The observed t_R are printed at the peak tops. Figure 2 has only 13 peaks from 25 standard esters, confirming, as was mentioned earlier, that esters with the same total number of carbon atoms were coeluted or poorly separated.

The forecasted t_R (by equation 20) are summarized in Table IV, and the experimental values are included for comparison. All the predicted t_R agree well with the experimental values. The highest differences were $\pm 1.4\%$ and $\pm 1.1\%$ for the programming rate of 2°C and 4°C, respectively.

Prediction of t_R of Beeswax in TPGC

Beeswax was used as a demonstration sample to which equation 20 can be applied to predict the $t_{R(\text{TPGC})}$ of natural wax esters. The wax was directly injected into the AT-5 capillary column without derivatization. Beeswax is a very complex mixture. Beside the esters of long chain alcohols and fatty acids, it also contains hydrocarbons, free fatty acids, and long chain alcohols. Figure 3 is part of the chromatograms of beeswax chromatographed from 260°C to 320°C at a temperature gradient of 2°C/min and 4°C/min, respectively. The initial hold times were set at 2 min for both chromatograms. The observed t_R are printed at the peak tops. The forecasted t_R were similarly calculated as the standard

esters in the previous section and summarized in Table V. Calculation began at the total carbon number of 38, as was reported by Aichhloz and Lorbeer (16) (i.e., that beeswax contains ester of 38 carbon atoms and higher). The carbon numbers of the tentatively identified peaks are printed at the peak top of the chromatograms. The differences between the experimental and predicted t_R values of both chromatograms are used as criteria for identification. The esters of 38, 40, 42, 44, and 46 carbon atoms were found in the beeswax. However, because beeswax is a very complex mixture (8–10,16), there are peaks that cannot be identified.

Conclusion

The proposed method, which extends from the linear free energy relationship, can be applied to predict the GC t_R of fatty acid esters of long chain alcohols in both isothermal and temperature-programmed conditions. The difference in the increment in free energy per carbon atom for the acid and alcohol is small. Therefore, esters of different numbers of the acid and alcohol, but with the same total number of carbon atoms, were poorly separated on the AT-5 column. Consequently, identification of individual esters can only be done by their total carbon numbers, unless GC is coupled with other identification tools (e.g., MS or another GC).

Table IV. $t_{R(\text{TPGC})}$ of Standard Fatty Acid Esters of Long Chain Alcohols, from 260°C to 320°C*

z_1/z_2	$z_1 + z_2$	$t_{R(\text{TPGC})}$ (2°C/min, $t_M = 0.627$)			$t_{R(\text{TPGC})}$ (4°C/min, $t_M = 0.628$)		
		$t_{R(\text{exp})}$	$t_{R(\text{cal})}$	% Δ^\dagger	$t_{R(\text{exp})}$	$t_{R(\text{cal})}$	% Δ
14/14	28	5.025	5.026	-0.20	4.834	4.810	0.41
15/14	29	6.073	6.058	0.16	5.694	5.670	0.35
16/14	30	7.334	7.267	0.82	6.684	6.624	0.90
17/14	31	8.715	8.657	0.69	7.705	7.664	0.52
15/16	31	8.715	8.702	0.23	7.705	7.691	0.13
18/14	32	10.373	10.227	1.35	8.862	8.776	0.90
16/16	32	10.373	10.250	1.16	8.862	8.784	0.90
14/18	32	10.373	10.368	0.00	8.862	8.853	0.11
17/16	33	12.055	11.962	0.83	9.967	9.934	0.40
15/18	33	12.055	12.058	0.00	9.967	9.984	-0.10
18/16	34	13.976	13.824	1.14	11.188	11.128	0.54
16/18	34	13.976	13.893	0.64	11.188	11.157	0.27
14/20	34	13.976	14.067	-0.64	11.188	11.242	-0.45
17/18	35	15.874	15.855	0.06	12.355	12.359	0.00
15/20	35	15.874	15.999	-0.82	12.355	12.422	-0.49
18/18	36	18.015	17.923	0.55	13.622	13.582	0.29
14/22	36	18.015	18.247	-1.28	13.622	13.711	-0.66
16/20	36	18.015	18.033	-0.06	13.622	13.622	0.00
17/20	37	20.086	20.149	-0.30	14.805	14.833	-0.20
19/18	37	20.086	20.076	0.05	14.805	14.816	-0.14
15/22	37	20.086	20.324	-1.14	14.805	14.896	-0.68
18/20	38	22.223	22.329	-0.50	16.002	16.050	-0.31
16/22	38	22.223	22.462	-1.08	16.002	16.088	-0.56
17/22	39	24.319	24.646	-1.36	17.131	17.287	-0.93
18/22	40	26.497	26.862	-1.36	18.430	18.630	-1.09

* The initial hold times are 2 min for both conditions.

† % $\Delta = (t_{R(\text{exp})} - t_{R(\text{cal})})/t_{R(\text{cal})}$.

Table V. $t_{R(TPGC)}$ of Beeswax from 260°C to 320°C*

z_i/z_j	$z_i + z_j$	$t_{R(TPGC)} (2^\circ\text{C}/\text{min}, t_M = 0.623)$			$t_{R(TPGC)} (4^\circ\text{C}/\text{min}, t_M = 0.623)$		
		$t_{R(\text{exp})}$	$t_{R(\text{cal})}$	% Δ *	$t_{R(\text{exp})}$	$t_{R(\text{cal})}$	% Δ
14/24	38	22.83	22.62	0.91	16.13	16.12	0.06
16/22	38	22.83	22.40	1.91	16.13	16.04	0.56
14/26	40	27.00	27.14	-0.52	18.53	18.67	-0.81
16/24	40	27.00	26.93	0.27	18.53	18.60	-0.43
18/22	40	27.00	26.79	0.77	18.53	18.57	-0.27
20/20	40	27.00	26.74	0.96	18.53	18.58	-0.32
14/26	40	27.62	27.14	1.74	18.96	18.67	1.54
16/26	42	31.27	31.43	-0.50	21.79	22.02	-1.10
18/24	42	31.27	31.32	-0.14	21.79	21.99	-0.96
20/22	42	31.27	31.27	0.01	21.79	22.01	-1.06
22/20	42	31.27	31.29	-0.07	21.79	22.07	-1.33
12/30	42	31.27	31.83	0.06	21.79	22.19	0.72
14/28	42	31.85	31.60	0.78	22.35	22.09	1.16
26/16	42	31.85	31.55	0.93	22.35	22.32	0.13
28/14	42	31.85	31.79	0.19	22.35	22.51	-0.72
14/30	44	36.08	36.63	-1.51	26.40	26.86	-1.74
16/28	44	36.08	36.46	-1.04	26.40	26.80	-1.52
18/26	44	36.08	36.35	-0.75	26.40	26.78	-1.44
20/24	44	36.08	36.31	-0.64	26.40	26.80	-1.52
14/30	44	36.08	36.63	0.55	26.40	26.86	0.85
16/28	44	36.08	36.46	1.01	26.40	26.80	1.07
18/26	44	36.08	36.35	1.30	26.40	26.78	1.14
20/24	44	36.83	36.31	1.40	27.09	26.80	1.07
22/22	44	36.83	36.35	1.31	27.09	26.88	0.78
24/20	44	36.83	36.45	1.02	27.09	27.01	0.30
26/18	44	36.83	36.63	0.53	27.09	27.20	-0.41
14/31	45	41.28	39.82	3.54	31.42	29.95	4.68
14/32	46	42.19	43.61	-3.38	32.29	33.63	-4.15
14/32	46	42.19	43.61	-0.37	32.29	33.63	-0.09
16/30	46	42.19	43.44	0.03	32.29	33.56	0.12
18/28	46	43.43	43.33	0.27	33.60	33.55	0.15
20/26	46	43.43	43.31	0.33	33.60	33.60	0.00
22/24	46	43.43	43.36	0.21	33.60	33.70	-0.30
24/22	46	43.43	43.50	-0.11	33.60	33.87	-0.80

* The initial hold times are 2 min for both conditions.

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