

Forecasting Retention Times of Fatty Acid Methyl Esters in Temperature-Programmed Gas Chromatography

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Abstract

A hypothetical equation is derived from a method of column slicing previously described. When the derived equation is used to forecast the temperature-programmed gas chromatographic (TPGC) retention times of fatty acid methyl esters (FAMES), the differences between the observed and calculated values are -2.6 to 3.4%. The greatest differences are found with higher programming rates and longer carbon chain lengths. When a flow-adjustment term is incorporated into the equation, the greatest difference between the observed and calculated retention times is reduced to approximately 2.7%. This equation can also accurately forecast the TPGC retention times of both saturated and unsaturated FAMES from cauliflower seed oil.

Introduction

Gas chromatographic (GC) identification of fatty acid methyl esters (FAMES) can be performed using several methods (1-6). The equivalent chain length (ECL) or equivalent carbon number (ECN) methods proposed by Miwa et al. (5) and Woodford and van Gent (6) are widely accepted for the tentative identification of FAMES. In practice, ECL values are generally determined under isothermal conditions. With the advent of capillary GC, the analysis of FAMES has become more and more complex, and isothermal capillary GC either does not resolve all FAMES or is too tedious in analytical practice. Therefore, temperature-programmed GC (TPGC) is more demanding. Methods of calculating temperature-programmed retention times or indices of organic compounds with high accuracy have been described in several papers (7-13).

In this paper, the equation by Krisnangkura et al. (14) is combined with the method of column slicing by Cavalli and Guinchard (12) to predict the retention times of FAMES using TPGC.

Experimental

Theory

The retention time (t_R) of a compound in a TPGC can be accurately predicted by the following equation (12):

$$t_R = \sum_{i=1}^m \frac{t_0}{m} \left(1 + e^{\frac{x}{T(t_{i-1})} + y} \right) \quad \text{Eq 1}$$

$$t_R = \sum_{i=1}^m \frac{t_0}{m} (1 + k^i) \quad \text{Eq 2}$$

where k^i is the retention factor, t_0 is dead time, m is the number of elements, and $T(t_{i-1})$ is the oven temperature when the solute enters into the i th element at time t_{i-1} . The variables x and y are thermodynamic parameters determined at isothermal conditions. It has been demonstrated that under isothermal conditions, the retention times of compounds of homologous series may be predicted from (14)

$$\ln k^i = a + bn + \frac{c}{T} + \frac{dn}{T} \quad \text{Eq 3}$$

and

$$k^i = e^{\left(a + bn + \frac{c}{T} + \frac{dn}{T} \right)} \quad \text{Eq 4}$$

where T is the absolute temperature and a , b , c , and d are thermodynamic parameters.

$$a = \frac{\Delta S^0}{R} + \ln \beta \quad \text{Eq 5}$$

$$b = \frac{\delta S}{R} \quad \text{Eq 6}$$

$$c = \frac{\Delta H^0}{R} \quad \text{Eq 7}$$

$$d = \frac{\delta H}{R} \quad \text{Eq 8}$$

β is the phase ratio of the column. ΔH^0 and ΔS^0 are standard molar enthalpy and entropy, respectively. δH and δS are the increments in enthalpy and entropy, respectively, with respect to

carbon number (n). Combining Eq 2 and Eq 4 results in

$$t_R = \sum_{i=1}^m \frac{t_0}{m} \left(1 + e^{\left(a + bn + \frac{c+dn}{\theta_i} \right)} \right) \quad \text{Eq 9}$$

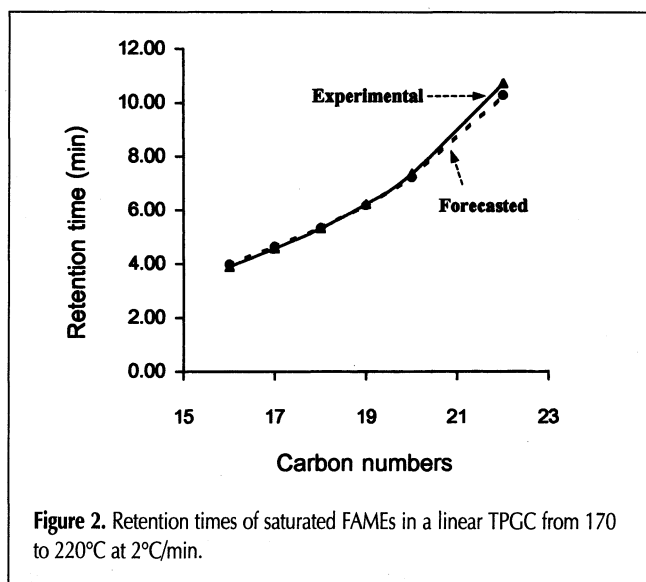
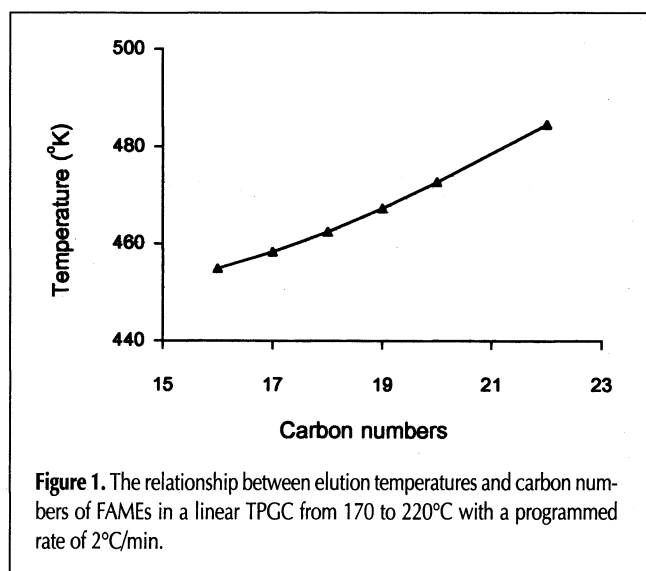
Eq 9 is an expansion of Eq 1, where θ_i is $T(t[i-1])$ and

$$x = a + bn \quad \text{Eq 10}$$

$$y = c + dn \quad \text{Eq 11}$$

Materials

FAMEs of various chain lengths were purchased from Sigma (St. Louis, MO). Cauliflower seed (*Brassica oleraceae*) was obtained from a plant grower. Transmethylation was carried out in situ with acid catalysis as described by Kalayasiri et al. (15).



GC

GC analysis was performed on a Shimadzu (Kyoto, Japan) model 14A GC equipped with a flame-ionization detector, split-splitless injector, and a C-R4A data processor. An Omegawax 250 (Supelco, Bellefonte, PA) capillary column (30 m × 0.25-mm i.d., 0.2-μm film thickness) was used with a nitrogen carrier gas flow rate of 0.7–1.5 mL/min. Injector and detector temperatures were set at 230°C.

Calculation

The four thermodynamic constants a , b , c , and d were determined under isothermal conditions as described in the literature (14) and have the numeric values -7.55 , -0.48 , 1496 , and 379 , respectively. The retention time of n -hexane was used as the t_0 in this study. These four constants and carbon numbers of fatty acids were converted to x and y values according to Eq 10 and 11, and the retention times were calculated as described by Cavalli and Guinchard (12) using a worksheet from Excel 8.0 (Microsoft, Redmond, WA).

Results and Discussion

Relationship between carbon numbers and retention times in TPGC

It was once reported that the relationship between retention time and carbon number of the members of a homologous series in linear TPGC is linear (7). On the contrary, this relationship is not linear (8,13). Krupcik and Bohov (8) also speculated that the relationship between carbon number and elution temperature would not be linear. The plots show that the relationship between temperatures and carbon numbers (Figure 1) and between retention times and carbon numbers (Figure 2) are not strictly linear. Also, Figure 2 shows that the predicted retention times calculated by Eq 9 are very close to the experimental values, but greater differences are found as the carbon number increases.

Table I demonstrates the fitness of the observed and predicted retention times of some FAMEs chromatographed at 170–220°C (hold time = 0). The forecasted retention temperatures are also included in Table I. At a temperature gradient of 2°C/min., all of the FAMEs are eluted in the gradient and confined to a linear TPGC. At other temperature gradients, some FAMEs are eluted at the ramp and are non-linear TPGC. The greatest difference between the observed and calculated values for the FAMEs of 22 carbons is 6.23% at the programmed rate of 10°C/min. As stated earlier, greater differences are usually observed for the FAMEs of higher carbon numbers and higher temperature gradients. The nature of the discrepancy is not known; however, some possible causes are listed here.

Carrier gas flow rate

The method used in this study does not adjust the carrier gas flow rate because of an increase in temperature. Generally, the carrier gas flow will decrease as temperature increases, and the observed values will be slightly higher. However, Cavalli and

Guinchard (16) pointed out that the adjustment of flow has very little effect on the predicted retention time.

Column phase ratio

Generally, an increase in temperature will decrease the mobile phase volume, which in turn will decrease the amount of solute in the mobile phase and result in a higher experimental value.

Column temperature

This discrepancy may arise from a slow rate of heat transfer

across the column wall. The actual column temperature is slightly lower than the oven temperature. This will also give rise to higher experimental values.

Accuracy in the determination of the four thermodynamic constants

Cavalli and Guinchard (12) mentioned that a small error in x and y of Eq 1 would have a pronounced effect on the predicted retention time.

Table I. Experimental and Forecasted TPGC Retention Times of Saturated FAMES of Different Carbon Chain Length Using Eq 9

CN	T	Eq. 9	t_R	Δ (%)
2°C/min ($t_0 = 0.945$)				
16	181.65	5.83	5.7	-2.28
17	185.01	7.51	7.38	-1.76
18	189.11	9.56	9.41	-1.59
19	193.89	11.95	11.82	-1.10
20	199.25	14.64	14.52	-0.83
22	211.13	20.57	20.59	0.10
4°C/min ($t_0 = 0.944$)				
16	190.27	5.07	4.96	-2.22
17	195.08	6.27	6.17	-1.62
18	200.55	7.64	7.55	-1.19
19	206.54	9.14	9.08	-0.66
20	212.88	10.73	10.72	-0.09
22	220.00	14.18	14.36	1.25
6°C/min ($t_0 = 1.000$)				
16	198.41	4.74	4.62	-2.60
17	204.13	5.69	5.58	-1.97
18	210.38	6.73	6.65	-1.20
19	216.98	7.83	7.78	-0.64
20	220.00	9.00	9.01	0.11
22	220.00	12.26	12.5	1.92
8°C/min ($t_0 = 0.948$)				
16	203.37	4.17	4.07	-2.46
17	209.50	4.94	4.86	-1.65
18	216.07	5.76	5.7	-1.05
19	220.00	6.63	6.62	-0.15
20	220.00	7.68	7.75	0.90
22	220.00	10.70	11.01	2.82
10°C/min ($t_0 = 0.999$)				
16	210.00	4.00	3.92	-2.04
17	216.55	4.66	4.59	-1.53
18	220.00	5.36	5.33	-0.56
19	220.00	6.21	6.24	0.48
20	220.00	7.27	7.39	1.62
22	220.00	10.38	10.75	3.44

* Abbreviations: T, elution temperature; Eq 9, forecasted retention time; t_R , experimental retention time; Δ , percent difference.

Table II. Experimental and Forecasted TPGC Retention Times of Saturated FAMES of Different Carbon Chain Length Using Eq 13

CN	T	Eq. 9	t_R	Δ (%)
2°C/min ($t_0 = 0.945$)				
16	181.47	5.74	5.7	-0.70
17	184.79	7.40	7.38	-0.27
18	188.85	9.43	9.41	-0.21
19	193.63	11.82	11.82	0.00
20	199.01	14.51	14.52	0.07
22	210.99	20.51	20.59	0.39
4°C/min ($t_0 = 0.944$)				
16	190.10	5.03	4.96	-1.41
17	194.91	6.23	6.17	-0.97
18	200.40	7.61	7.75	-0.79
19	206.45	9.12	9.08	-0.44
20	212.88	10.73	10.72	-0.09
22	220.00	14.24	14.36	0.84
6°C/min ($t_0 = 1.0$)				
16	198.32	4.72	4.62	-2.16
17	204.08	5.68	5.58	-1.79
18	200.40	7.61	7.75	-0.79
19	217.10	7.85	7.78	-0.90
20	220.00	9.05	9.01	-0.44
22	220.00	12.36	12.5	1.12
8°C/min ($t_0 = 0.948$)				
16	203.40	4.18	4.07	-2.70
17	209.60	4.95	4.86	-1.85
18	216.29	5.79	5.7	-1.58
19	220.00	6.68	6.62	-0.91
20	220.00	7.75	7.75	0.00
22	220.00	10.83	11.01	1.63
10°C/min ($t_0 = 0.998$)				
16	210.14	4.02	3.92	-2.55
17	216.80	4.68	4.59	-1.96
18	220.00	5.40	5.33	-1.31
19	220.00	6.27	6.24	-0.48
20	220.00	7.35	7.39	0.54
22	220.00	10.50	10.75	2.33

* Abbreviations: T, elution temperature; Eq 13, forecasted retention time; t_R , experimental retention time; Δ , percent difference.

Carrier gas flow rate and the adjustment of t_0

Among the aforementioned four possible causes, carrier gas flow rate is the easiest one to correct on the spreadsheet. Normally, carrier gas viscosity increases as temperature increases. Thus, carrier gas flow rate decreases as the head pressure remains constant. Injections of hexane at isothermal temperatures between 150 and 220°C show that t_R increases linearly with oven temperature at a rate of 0.0013 min/°C (g) (Figure 3). Incorporating this incremental term to Eq 9 results in

$$t_R = \sum_{i=1}^m \frac{t_0 [1+g(\theta_i - T_i)]}{m} \left(1 + e^{\left(a + bT_i + \frac{c+dT_i}{\theta_i} \right)} \right) \quad \text{Eq 12}$$

$$t_R = \sum_{i=1}^m \frac{t_m}{m} \left(1 + e^{\left(a + bT_i + \frac{c+dT_i}{\theta_i} \right)} \right) \quad \text{Eq 13}$$

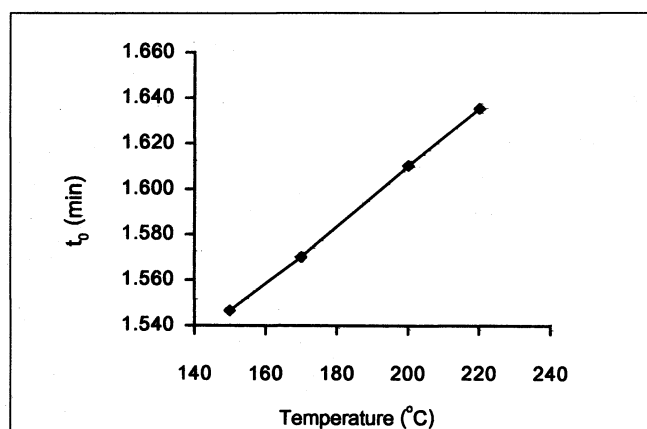


Figure 3. Change in retention times of *n*-hexane with oven temperatures. The head pressure was held constant.

$$t_m = t_0 [1+g(\theta_i - T_i)] \quad \text{Eq 14}$$

T_i is initial temperature. t_m is generated in column D as shown in the spreadsheet (Figure 4). Table II shows that better agreement is found between the observed and forecasted retention times of saturated FAMES. The greatest difference is reduced to about 2.7%.

Forecasting the retention times of natural FAMES

Figure 5 is a chromatogram of cauliflower seed oil methyl esters chromatographed from 170 to 220°C at a temperature gradient of 2°C/min. Peaks are labeled with retention times in minutes. The observed retention times and the predicted values are listed in Table III. In addition, Table III includes the retention times of experimental data for the programmed rate of 4°C/min. The greatest differences between the observed and predicted retention times for the programmed rates of 2°C/min and 4°C/min are 2.9% and 3.2%, respectively. Although the discrepancy between the observed and forecasted retention times are within instrumental error, it should be noted that the difference tends to shift to the more positive value, that is, the slope of the calculated line is always lower than the observed values. Thus, it is believed that there is another influence, (e.g., heat transfer across the column wall) that slows the migration of solutes in the column.

Conclusion

Results in this study show that the retention times of both saturated and unsaturated FAMES chromatographed under a linear or non-linear TPGC can be accurately predicted. Furthermore, it is speculated that the modified equation described in this study may be applicable to multi-step TPGC as well. The predicted retention times tend to be lower for higher-retained compounds. It is suggested that a column of high mass

Table III. Experimental and Forecasted Retention Times of Cauliflower Seed Oil Methyl Esters using Eq 13

FAMES	ECL*	Oven temperature 170 to 220°C (2°C/min, $t_0 = 1.579$)				Oven temperature 170 to 250°C (4°C/min, $t_0 = 1.588$)			
		<i>T</i>	t_R	Eq 13	Δ (%)	<i>T</i>	t_R	Eq 13	Δ (%)
Methyl palmitate	16.00	187.42	8.71	8.67	-0.48	199.23	7.37	7.28	-1.25
Methyl palmitoleate	16.18	188.15	9.07	—	—	200.33	7.58	—	—
Methyl heptadecanoate	17.00	191.80	10.90	10.89	-0.07	205.15	8.79	8.75	-0.51
Methyl stearate	18.00	196.90	13.45	13.475	0.19	211.50	10.37	10.36	-0.10
Methyl oleate	18.18	197.88	13.94	14.05	0.78	212.68	10.67	10.74	0.62
Methyl linoleate	18.59	200.19	15.10	15.39	1.88	215.42	11.35	11.56	1.79
Methyl linolenate	19.21	203.85	16.92	17.35	2.46	219.64	12.41	12.72	2.41
Methyl arachidate	20.00	208.72	19.36	—	—	225.09	13.77	—	—
Methyl eicosa-11-enoate	20.18	209.86	19.93	20.13	0.99	226.34	14.04	14.26	1.52
Methyl behenate	22.00	220.00	25.85	—	—	238.91	17.23	—	—
Methyl erucate	22.18	220.00	26.49	26.97	1.76	240.14	17.53	17.92	2.17
Methyl linocerate	24.00	220.00	34.78	—	—	250.00	20.58	21.08	2.39
Methyl tetracosenoate†	24.18	220.00	35.83	36.91	2.93	250.00	20.89	21.59	3.23

* ECL, equivalent chain lengths from references 14 and 17; *T*, temperature; t_R , experimental retention time; Eq 13, predicted retention time; Δ , percent difference.

† Tentative identification as 24:1.

	A	B	C	D	E	F	G	H	I	J	K
	Position	Temp	tr	tr _n		T _i	T _f	Hold time	m	Length	
2	0	443.00	0	1.5860		443	523	0	1000	1000	
3	1	443.05	0.012	1.5861		Gradient		x	y	t ₀	g
4	2	443.09	0.023	1.5861		4	7560.00	-15.230	1.586	0.0013	
5	3	443.14	0.035	1.5862		a	b	c	d	n	
6	4	443.18	0.048	1.5862		-7.55	-0.48	1496	378	18	
7	5	443.23	0.057	1.5863		=t ₀ +g*(B2-T _i)		=C1002+tm/m*(1+Exp((B1002+y)))			
8	6	443.28	0.069	1.5864		temp (K)		temp (C)	tr		
9	7	443.32	0.080	1.5864		472.31	199.31	7.33			
10	8	443.37	0.092	1.5865							
1001	999	472.29	7.322	1.8241							
1002	1000	472.31	7.327	1.8241							

Figure 4. Excel 8.0 worksheet for the calculation of TPGC retention time of FAMEs according to Eq 13. The boxes with arrows show the formula of the cells. T_i and T_f are the initial and final oven temperatures, respectively. t_0 and t_m are the retention times of *n*-hexane at T_i and θ_i , respectively. Column length is arbitrarily set at 1000.

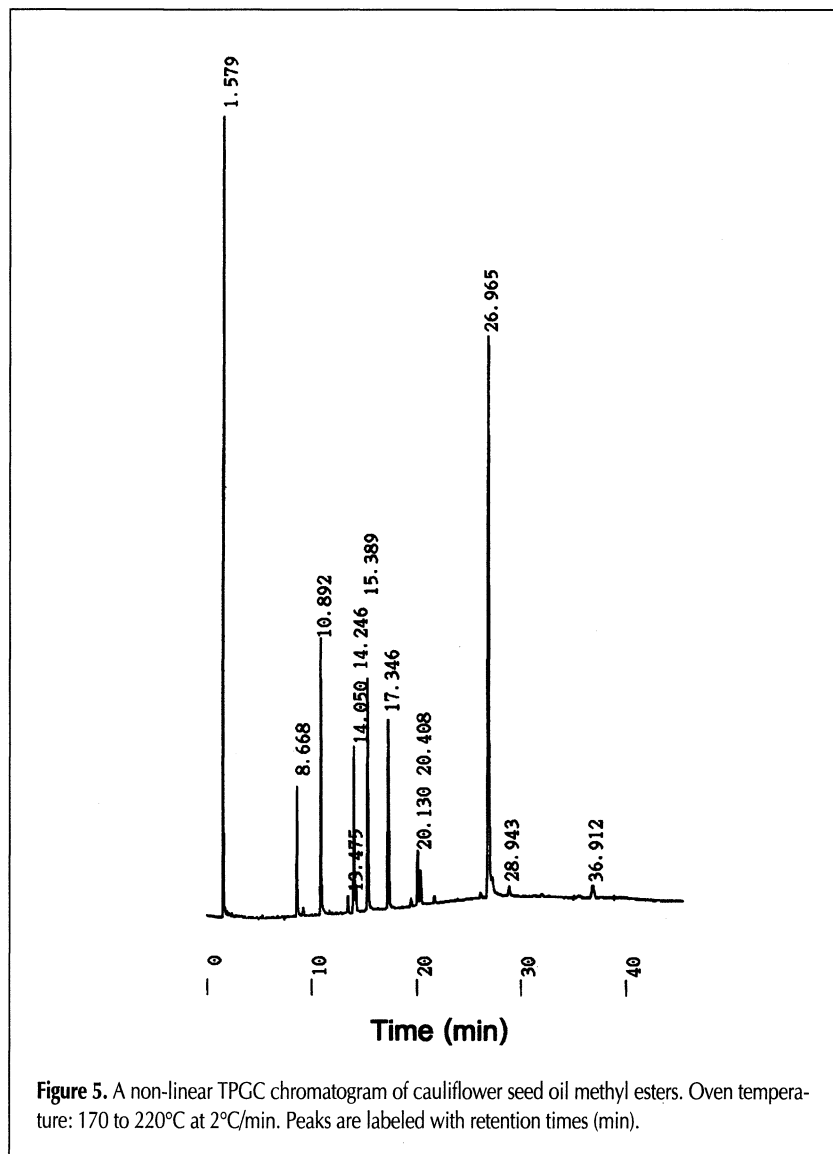


Figure 5. A non-linear TPGC chromatogram of cauliflower seed oil methyl esters. Oven temperature: 170 to 220°C at 2°C/min. Peaks are labeled with retention times (min).

transfer (e.g., aluminum clad capillary) may reduce the differences between the observed and calculated values.

Acknowledgments

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