

An Alternative Approach for the Estimation of Equivalent Temperature in Gas Chromatography

Kornkanok Aryusuk, Dararat Yensruang, and Kanit Krisnangkura*

Biochemical Technology Division, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi. Toongkru, Bangkok 10140 Thailand.

Abstract

The temperature at which the isothermal retention time ($t_{R(iso)}$) is equal to the temperature-programmed retention time ($t_{R(TPGC)}$) or the isothermal retention index (I_{iso}) is equal to the temperature-programmed retention index (I_{TPGC}) is defined as the equivalent temperature (T_{eq}). The T_{eq} of one-, two-, three-, and four-step temperature-programmed gas chromatography (TPGC) of unsaturated fatty acid methyl esters (FAMES) from Chinese mustard seed oil is calculated. All of the t_R values obtained at the T_{eq} ($t_{R(Teq)}$) are very close to the $t_{R(TPGC)}$. The highest difference for each chromatogram is less than 0.5%. The slight deviation may partly arrive from the difficulty in setting the desired carrier gas flow rate. Also, good agreement among equivalent chain length values determined by different methods is observed, including the graphical method at the T_{eq} . Therefore, it is speculated that the proposed method may facilitate the GC identification of FAMES as well as other organic compounds in TPGC by using the available isothermal retention index database.

Introduction

Kovats retention index (I) (1) and equivalent chain length (ECL) (2,3) have widely been accepted as identification tools for organic compounds in general and for fatty acid methyl esters (FAMES), respectively. Both values are closely related and convertible (4). At present, the accuracy in the determination of I can be improved to 0.1 unit or better (5). However, one of the major drawbacks of using I and ECL in identification is they are temperature dependent and their values tend to change with temperature. The temperature dependence of I had been discussed in the literature (6,7). Therefore, both systems are limited to isothermal gas chromatography (GC). However, today complex mixtures are usually analyzed under temperature-programmed conditions. Thus a similar system is necessary for the identification of organic compounds in temperature-programmed GC (TPGC). Van den Dool and Kratz (8) were the first to propose an equation for the calculation of retention index in TPGC (I_{TPGC}) from the elution

temperature (T_R) or the retention time (t_R) (or both):

$$I_{TPGC} = 100z + 100 \frac{T_{R(x)} - T_{R(z)}}{T_{R(z+1)} - T_{R(z)}} \quad \text{Eq. 1A}$$

$$I_{TPGC} = 100z + 100 \frac{t_{R(x)} - t_{R(z)}}{t_{R(z+1)} - t_{R(z)}} \quad \text{Eq. 1B}$$

T_R is the elution temperature and z is the carbon number of the n -paraffin eluting before the compound of interest. The subscript x indicates the solute being measured.

The validity of equation 1B depends on the linear relationship between T_R and t_R in equation 2 and it is limited to linear TPGC without an isothermal step.

$$T_R = T_i + rt_R \quad \text{Eq. 2}$$

where r is the heating rate and T_i is the initial temperature.

The utility of I_{TPGC} depends very much on its reproducibility, which in turn depends on T_i , r , and the carrier gas velocity and pressure (6). Thus, it is very difficult to reproduce I_{TPGC} between different laboratories unless all the chromatographic conditions are reported (15,16). Consequently, several nonlinear equations were proposed for its calculation (9–14).

At the same time, several researchers tried to find the T_{eq} where $t_{R(iso)} = t_{R(TPGC)}$ or $I = I_{TPGC}$ (17–20). Although, all the proposed methods were empirical or semiempirical and they were not suitable for use as identification aids, the concept behind these developments was very interesting. If this goal could be achieved, one could make use of the widely accepted and largely accumulated isothermal retention index values for TPGC identification.

I is defined in purely thermodynamic parameters, but I_{TPGC} depends on fluid dynamic variables as well as thermodynamic parameters (21). With thorough theoretical analysis, González and Nardillo (16) concluded that I_{TPGC} depends not only on the thermodynamic parameters but also on the fluid dynamic elution process. Therefore, it was not possible to define I_{TPGC} in purely thermodynamic parameters (16) and I_{TPGC} cannot be directly converted to I unless all the fluid dynamic variables are carefully defined (21). This might be the major obstruction, which hin-

* Author to whom correspondence should be addressed: email kanitksnk@yahoo.com.

dered the general acceptance of I_{TPGC} compared with that of I . Many researchers focused on the methods of predicting the $t_{\text{R(TPGC)}}$ from I using the thermodynamic parameters at the specific fluid dynamic conditions (21–31).

As mentioned earlier, if T_{eq} of TPGC is known, one could make use of the large database of I and ECL in the identification of organic compounds in general and particularly FAMES. Thus, a method for the calculation of T_{eq} is proposed and we are very optimistic that with the recent advancement in the methods of calculation of $t_{\text{R(TPGC)}}$ from I , or vice versa, accurate T_{eq} values can be determined.

The standard free energy of solution (ΔG°) of compounds in the same homologous series can be calculated from equation 3 (32).

$$\Delta G^{\circ} = \Delta G_0 + z\delta G \quad \text{Eq. 3}$$

where ΔG_0 is the free energy of the hypothetical molecule with zero carbon atom, δG is the free energy increment per carbon atom and z is the number of carbon atoms.

From basic thermodynamics:

$$\Delta G = \Delta H - T\Delta S \quad \text{Eq. 4}$$

$$\text{and } \ln k = \frac{-\Delta G^{\circ}}{RT} - \ln \beta \quad \text{Eq. 5}$$

where ΔH and ΔS are the change in enthalpy and entropy of solution, respectively. T is the absolute temperature, k is the retention factor, and β is the column phase ratio; R is the gas constant. Equation 6 is obtained by substituting equation 4 into equation 3.

$$\Delta G^{\circ} = \Delta H_0 - T\Delta S_0 + z\delta H_0 - zT\delta S_0 \quad \text{Eq. 6}$$

where ΔH_0 and ΔS_0 are the enthalpy and entropy of solution of the hypothetical molecule with zero carbon atom, and δH and δS are the increments in enthalpy and entropy per carbon atom, respectively.

Substituting equation 6 into equation 5, equation 7 is obtained.

$$\ln k = \alpha + bz + \frac{c}{T} + \frac{dz}{T} \quad \text{Eq. 7A}$$

$$\text{or } \ln \frac{t_{\text{R}} - t_{\text{M}}}{t_{\text{M}}} = \alpha + bz + \frac{c}{T} + \frac{dz}{T} \quad \text{Eq. 7B}$$

where t_{M} is the gas hold-up time and :

$$\alpha = \frac{\Delta S_0}{R} - \ln \beta \quad \text{Eq. 8}$$

$$b = \frac{\delta S}{R} \quad \text{Eq. 9}$$

$$c = \frac{\Delta H_0}{R} \quad \text{Eq. 10}$$

$$d = \frac{\delta H}{R} \quad \text{Eq. 11}$$

Rearranging equation 7A, equation 12 is obtained.

$$T_{\text{eq}} = \frac{c + dz}{\ln k - \alpha - bz} \quad \text{Eq. 12}$$

where T_{eq} indicates that the experiment is performed at the equivalent temperature.

Equation 7 is slightly different from most of the equations cited previously in that they are just derived by combining equations 3–5. Therefore, there are certain merits and limitations of using equation 7 for the calculation of I or t_{R} in the isothermal and TPGC. It has been known that equation 3 is not strictly linear over a wide range of z (33,34), thus it is speculated that the applicability of equation 7 will be less accurate outside the range of z that is used to determine the constants (a , b , c , and d).

Experimental

Materials

Fatty acid methyl esters were purchased from Sigma Chemical Co. (St. Louis, MO). Chinese mustard seed (*Brassica juncea*) was obtained from the grower's shop. Transmethylation of plant seed oils was carried out *in situ* with acid catalysis as described by Kalayasiri et al. (35).

GC

GC analysis was performed on a Shimadzu model 14A GC. The instrument was equipped with a flame-ionization detector, split-splitless injector, a C-R4A data processor (Shimadzu, Kyoto, Japan) and a 70% cyanopropyl polysiloxane (BPX-70, SGE Pty Ltd., Ringwood, Australia.) capillary column (30-m \times 0.25-mm i.d., 0.25- μm film thickness). Nitrogen was used as the carrier gas and its flow rate was controlled by a mechanical pressure gauge. Injector and detector temperatures were set at 230°C. In order to get a consistent injection time, a remote start for the data processor has been attached to the syringe handle. Temperature-programmed conditions are reported in Table I.

Determination of the four column constants

The four column constants of equation 7 for FAME were determined as described by Krisnangkura et al. (36). At constant T , equation 7A is reduced to equation 13:

$$\ln k = \alpha' + b'z + \quad \text{Eq. 13}$$

Table I. Temperature-Programmed Conditions*

Program	Step	Initial hold time (min)	Initial T (°C)	Final T (°C)	Rate (°C/min)
A (one step)	1	2	160	220	2
B (two step)	1	2	160	190	2
	2	1	190	220	4
C (three step)	1	2	160	180	2
	2	1	180	200	4
	3	1	200	220	6
D (four step)	1	2	160	170	2
	2	1	170	180	3
	3	1	180	190	4
	4	1	190	220	5

* Injector and detector temperatures were 250°C.

$$\text{where } \alpha' = \alpha + \frac{c}{T} \quad \text{Eq. 14}$$

$$\text{and } b' = b + \frac{d}{T} \quad \text{Eq. 15}$$

The a' and b' (equation 13) are the intercept and slope, respectively, of the $\ln k' - z$ plot. The values of a , c , b , and d are obtained by plotting α versus $1/T$ (equation 14) and b' versus $1/T$ (equation 15), respectively. It should be pointed out that higher decimal number of the four constants would yield more accurate values of the k' and t_R values. Therefore, unrounded values of four constants were generally used. We also observed that the remote start switch attached to the syringe handle tended to reduce human error and produced more consistent injection points. Consequently, higher R^2 values were obtained for the $\alpha' - 1/T$ and $b' - 1/T$ plots. It is speculated that an autosampler would also give more consistent injection points.

Results

Calculation of equivalent temperature

Because BPX-70 is very polar and the column is operated at high temperatures, hexane is almost not retained; the t_R of hexane at 160°C or higher temperature is very close to t_M , calculated according to Wattanachaiyong et al. (37). Thus, using t_R of hexane ($t_{R(\text{hex})}$) as the t_M value in equation 7 does not introduce great error but when hexane is used as the solvent its t_R value is easily obtained from the chromatogram. However, hexane is still retained appreciably in a nonpolar column (e.g., 100% polydimethylsiloxane) at this temperature, therefore hexane cannot be used as the t_M . Equation 16 is obtained by substitution these four numeric constants into equation 12 and $\ln k'$ is expanded:

$$T_{\text{eq}} = \frac{2272.36 + 356.09 \text{ECL}_{(T_{\text{eq}})}}{\ln \left(\frac{t_R - t_{M(T_{\text{eq}})}}{t_{M(T_{\text{eq}})}} \right) + 9.839 + 0.487 \text{ECL}_{(T_{\text{eq}})}} \quad \text{Eq. 16}$$

Subscript (T_{eq}) in equation 16 is simply to stress that experiment and calculation are performed at the equivalent temperature and when FAMES are used as references, z can be replaced by ECL. Theoretically, ECL in TPGC can be calculated by any methods mentioned in the literature (22–31), but we are more familiar with the column slicing method of Callvalli and Guinchard (28,29), which was modified by Kittiratanapiboon et al. (30) and Lomsugarit et al. (38), as described in equation 17:

$$t_R = \sum_{i=1}^m \frac{t_M [1 + g(\theta_i - T_i)]}{m} \left(1 + e^{(-9.839 - 0.487Z + \frac{2272.36}{\theta_i} + \frac{356.09Z}{\theta_i})} \right) \quad \text{Eq. 17}$$

where g is the change in t_M /change in T , m (=1000) is the number of columns being arbitrarily divided (28,38) and θ_i is the temperature of the i^{th} element (arbitrary column).

When $\text{ECL}_{(\text{TPGC})}$ is obtained (from equation 17), it is substituted into $\text{ECL}_{(\text{Teq})}$ in equation 16. T_{eq} is defined here as the temperature at which isothermal $t_R = t_{R(\text{TPGC})}$ and isothermal ECL (or I) = $\text{ECL}_{(\text{TPGC})}$ (or I_{TPGC}), thus at the T_{eq} , $\text{ECL}_{(\text{Teq})}$ can be replaced by $\text{ECL}_{(\text{TPGC})}$ and t_R can be replaced by $t_{R(\text{TPGC})}$. However, the value of $t_{M(\text{Teq})}$ is still not known but it can be solved by two iteration cycles, (higher iterative number does not yield much better accuracy). Initially the t_M of the initial temperature ($t_{M(T_i)}$) is used. The obtained $T_{\text{eq}(1)}$ will not be the actual T_{eq} , but it is very close to the T_{eq} . Substitution of the $T_{\text{eq}(1)}$ into equation 18 will give $t_{M(\text{Teq})}$.

$$t_{M(T_{\text{eq}})} = t_{M(T_i)} + g(T_{\text{eq}} - T_i) \quad \text{Eq. 18}$$

Equation 18 is the temperature correction for t_M adopted from equation 17. The calculated $t_{M(\text{Teq})}$ is then substituted back into equation 16 and a closer value of T_{eq} is obtained.

Figure 1 is the temperature-programmed GC of FAMES derived from Chinese mustard seed oil. The chromatographic conditions of each subchromatogram are summarized in Table I. Only the calculated ECL values of unsaturated FAMES are listed in Table II. Also, the calculated T_{eq} values of the unsaturated FAMES in each

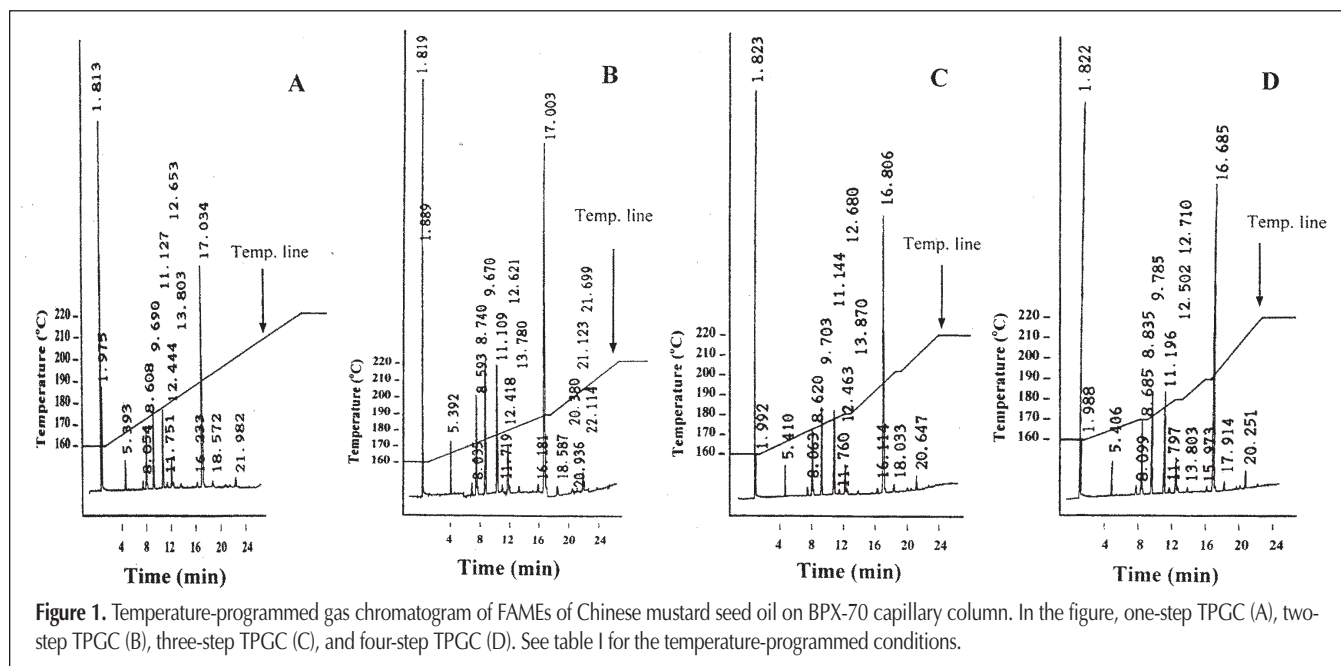


Figure 1. Temperature-programmed gas chromatogram of FAMES of Chinese mustard seed oil on BPX-70 capillary column. In the figure, one-step TPGC (A), two-step TPGC (B), three-step TPGC (C), and four-step TPGC (D). See table I for the temperature-programmed conditions.

subchromatogram are summarized in the same Table. The $t_{R(\text{TPGC})}$ values of each FAME in each chromatogram are varied slightly according to the chromatographic conditions and carrier gas flow rate. The greatest change in the $t_{R(\text{TPGC})}$ value (in Table II) is 1.73 min for FAME of 24:1, and the change in T_{eq} for this FAME is approximately 1.8°C. The change in the ECL value calculated by equation 17 is 0.04 unit. It is speculated that with wider temperature programming range, the difference in the mentioned values would be higher and identification would also be more difficult without the T_{eq} as a point of reference.

Verification of the calculated equivalent temperature

To verify the validity of the calculated T_{eq} , GC conditions are set isothermally at the T_{eq} and $t_{M(\text{Teq})}$ of each unsaturated FAME. Actually, only the temperature was set, whereas the pressure gauge, which regulated the carrier gas flow, was untouched (constant pressure). The carrier gas flow through the capillary column is automatically adjusted as the oven temperature changed. Table III summarizes the values of T_{eq} , as well as the calculated t_M ($t_{M(\text{cal})}$), experimental t_M ($t_{M(\text{exp})}$), $t_{R(\text{TPGC})}$, and experimental ($t_{R(\text{Teq})}$) values of unsaturated FAMES. Good agreements are found between $t_{R(\text{Teq})}$ and $t_{R(\text{TPGC})}$. The highest difference is 0.49% for C_{24:1}. Only approximately 10% of the data have the difference values greater than 0.40%. The deviation may partly arise from the difficulty in obtaining the carrier gas flow rate for a predetermined value of t_M .

Comparison of equivalent chain length

In this section, the ECL values of FAMES determined by various methods are compared. These include the ECL values calculated according to equation 17 ($ECL_{(\text{TPGC})}$), the graphical method ($ECL_{(\text{G})}$) of Woodford and van Gent (2) and Miwa et al. (3) and the calculated method according to equation 19 [modified from the retention index (6)]:

$$ECL_{(T_{\text{eq}})} = ECL_{T_i} + (T_{\text{eq}} - T_i) \times \frac{\Delta ECL}{\Delta T} \quad \text{Eq. 19}$$

where $\Delta ECL/\Delta T$ is the change in ECL per change in T . Results are

summarized in Table IV. All the ECL values calculated by different methods are very close. Suggesting any ECL values can be used as an identification aid.

Although T_i and ECL_{T_i} are used as the references in equation 19, higher accuracy can be obtained if the temperature at which the last saturated FAME eluted prior to the interested FAME and if its ECL is used as the reference. This will narrow the interpolation range and minimize error caused by nonlinearity of $\Delta ECL/\Delta T$.

Analysis of the data of Lomsugarit (38)

Lomsugarit et al. have reported multistep TPGC retention data of FAMES from Chinese mustard seed oil on SA-WAX (Carbowax 20M), including the calculated $ECL_{(\text{TPGC})}$, but they were not aware that T_{eq} could be generated from the available data (38). Therefore, there were no experimental data at the reported T_{eq} . However, the ECL values at 160°C, 180°C, 190°C, and 210°C were reported together with $\Delta ECL/\Delta T$ for unsaturated FAMES. These available data can be used to estimate the T_{eq} and $ECL_{(\text{Teq})}$. Substitution of the available $ECL_{(\text{TPGC})}$, $t_{R(\text{TPGC})}$, and $t_{M(\text{Teq})}$ into equation 16 with the combination of equation 18, the T_{eq} is solved as described previously (see the Calculation of equivalent temperature section). The g value of their column was 0.0013 min/°C. The calculated T_{eq} values are summarized in Table V.

The $ECL_{(\text{TPGC})}$ and ECL_{Teq} values are only slightly different for unsaturated FAMES of 18 carbon atoms, but the differences are higher for higher carbon numbers. The highest differences between $ECL_{(\text{TPGC})}$ and $ECL_{(\text{Teq})}$ in Table V is 0.13 unit for 24:1. The difference is not very high, in this case, to induce incorrect identification. As pointed out earlier, ECL values of unsaturated FAMES tend to change with temperature and the difference in the ECL values, for 18:3 ($n = 3$) and some other FAMES, are as high as 0.3 unit in the temperature-programmed range. Thus identification would be ambiguous without the T_{eq} as the reference temperature. On the other hand, a library of $ECL_{(\text{TPGC})}$ can be built up to help identification. The proposed T_{eq} has a certain advantage that the isothermal ECL can be used for identification.

Table II. T_{eq} and $z_{(\text{TPGC})}$ of Unsaturated FAMES of Chinese Mustard Seed Oil Chromatographed at Different Temperature-Programmed Conditions*

A ($t_M = 1.813$ min)			B ($t_M = 1.819$ min)			C ($t_M = 1.823$ min)			D ($t_M = 1.822$ min)			Tentative identification†
$t_{R(\text{TPGC})}$	$z_{(\text{TPGC})}$	$T_{\text{eq}}(^{\circ}\text{C})$	$t_{R(\text{TPGC})}$	$z_{(\text{TPGC})}$	$T_{\text{eq}}(^{\circ}\text{C})$	$t_{R(\text{TPGC})}$	$z_{(\text{TPGC})}$	$T_{\text{eq}}(^{\circ}\text{C})$	$t_{R(\text{TPGC})}$	$z_{(\text{TPGC})}$	$T_{\text{eq}}(^{\circ}\text{C})$	
8.608	18.36	165.13	8.593	18.34	165.13	8.620	18.34	165.11	8.685	18.35	164.95	18:1(n-9)
9.690	18.97	166.22	9.670	18.95	166.22	9.703	18.96	166.26	9.785	18.95	165.95	18:2(n-6)
11.127	19.72	167.78	11.109	19.69	167.70	11.114	19.70	167.75	11.196	19.70	167.62	18:3(n-3)
12.444	20.35	169.21	12.418	20.32	169.14	12.463	20.33	169.18	12.502	20.33	169.09	20:1(n-9)
13.803	20.96	170.70	13.780	20.94	170.68	13.870	20.94	170.58	13.803	20.95	170.75	20:2(n-6)
17.034	22.34	174.51	17.003	22.31	174.44	16.806	22.32	174.81	16.685	22.32	174.96	22:1(n-9)
21.982	24.33	180.69	21.699	24.29	180.80	20.647	24.32	182.04	20.251	24.33	182.49	24:1(n-9)

* T_{eq} and $z_{(\text{TPGC})}$ are calculated according to equations 16 and 17, respectively. A, B, C, and D are the chromatographic conditions listed in Table I.

† The first group of number is the number of carbon atoms of the fatty acid, the number after the colon is the number of double bonds and the number in parenthesis is the position of the double bond counting from the methyl end (n) of the acid. The ECL values are taken from <http://www.SGE.com> (unrevel FAMES using ECL numbers and a 70% bis-cyanopropyl capillary column).

Discussion

Equation 12 is proposed here for the calculation of T_{eq} which is used as the reference temperature to the TPGC. In identification, the $ECL_{(TPGC)}$ (or I_{TPGC}) is required. The correctness in the identification depends very much on the accuracy in the determination of ECL_{TPGC} (or I_{TPGC}). As it was mentioned previously, there are many accurate methods for predicting $t_{R(TPGC)}$ from the thermodynamic parameters and any method of determination of ECL_{TPGC} (or I_{TPGC}) can be used in conjunction

with equation 12. At present, most of the methods are used for predicting $t_{R(TPGC)}$. In predicting $t_{R(TPGC)}$, both the entropic and enthalpic constants for each molecule must be known. Therefore, to calculate the ECL_{TPGC} (or I_{TPGC}) from $t_{R(TPGC)}$ of an unknown would not be possible, unless some modification is done to obtain these two constants. This is an advantage of equation 7 and 17, in which the entropic and enthalpic terms are expanded by including equation 3. The four thermodynamically related constants are derived from the distribution constant (K) and the column phase ratio (β). They are characteristic of the individual column. The change in column inside diameter or film thickness would undoubtedly affect the numeric values of the constants. Also, any change in the chemistry of the stationary phase would change the numeric values of the constants. This may include the same stationary phase but of different degree of deterioration either by excessive bleeding or oxidation. Molecular weight distribution of the stationary phase may also affect K and consequently may change the numerical values of the constants.

The four constants in equation 7 or equation 17 are derived by expansion of the entropic and enthalpic terms and are not independent of the temperature. Similarly, the β is not independent of the temperature. Equation 3 is not strictly linear. Therefore, all these weak points must be associated with equations 7 and 17, but all the numerical values are the average of seven different temperatures (170–200°C) and seven different carbon lengths (16–22). Thus, these equations are best used at these boundaries. Prediction outside these boundaries is possible but it may give less accurate results.

Koppenhoefer et al. (39) have demonstrated that the effect of T on t_M is complex, and at least three different nonlinear equations can be used to describe their relationships. However, Kittiratanapiboon et al. (30) showed that, within the experimental temperature range, t_M increases linearly with T , and a correction factor similar to equation 18 was incorporated into equation 17. However, many researchers dealing with TPGC are still unhappy or in doubt about the linearity of the t_M - T plot. Therefore, data reported by Koppenhoefer et al. (39) are re-analyzed using equation 20, one of the three equations proposed by Koppenhoefer et al.:

$$t_M = AT^B \quad \text{Eq. 20}$$

A and B are constants and have the values of $A = 0.80$ and $B = 0.64$ for H_2 at an inlet pressure of 0.5 bar.

The plot between the calculated t_M and T values (at temperatures between 50°C and 180°C) gives a straight line with the slope = 0.0596, intercept = 29.187, and $r^2 = 0.9997$, whereas the plot between the experimental t_M and T values gives a straight line with the slope = 0.0592, intercept = 29.162, and $r^2 = 0.9981$. These re-analysis results suggest that it is also possible to transform the experimental data of Koppenhoefer et al. (39) to a linear equation similar to the carrier gas flow correction factor reported by Kittiratanapiboon et al. (30).

Therefore, the correction factor of Kittiratanapiboon et al. (30) was adopted in this study because the g values can be simultaneously determined with the four column constants (a , b , c , and d).

Table III. Comparison of $t_{R(Teq)}$ and $t_{R(TPGC)}$ of Unsaturated FAMES of Chinese Mustard Seed Oil

One step (A)						
FAMES*	T_{eq}	$t_{M(cal)}$	$t_{M(exp)}$	$t_{R(TPGC)}$	$t_{R(Teq)}$	%Error
18:1 ($n-9$)	165.13	1.821	1.828	8.608	8.607	0.01
18:2 ($n-6$)	166.22	1.822	1.836	9.690	9.688	0.02
18:3 ($n-3$)	167.78	1.825	1.847	11.127	11.145	-0.16
20:1 ($n-9$)	169.21	1.827	1.850	12.444	12.467	-0.18
20:2 ($n-6$)	170.70	1.829	1.859	13.803	13.789	0.10
22:1 ($n-9$)	174.51	1.835	1.862	17.034	17.099	-0.38
24:1 ($n-9$)	180.69	1.844	1.879	21.982	21.874	0.49
Two step (B)						
FAMES*	T_{eq}	$t_{M(cal)}$	$t_{M(exp)}$	$t_{R(TPGC)}$	$t_{R(Teq)}$	%Error
18:1 ($n-9$)	165.13	1.827	1.828	8.593	8.611	-0.21
18:2 ($n-6$)	166.22	1.828	1.833	9.670	9.652	0.19
18:3 ($n-3$)	167.70	1.831	1.847	11.109	11.145	-0.32
20:1 ($n-9$)	169.14	1.833	1.842	12.418	12.454	-0.29
20:2 ($n-6$)	170.68	1.835	1.857	13.780	13.807	-0.20
22:1 ($n-9$)	174.44	1.841	1.862	17.003	17.051	-0.28
24:1 ($n-9$)	180.80	1.850	1.879	21.699	21.772	-0.34
Three step (C)						
FAMES*	T_{eq}	$t_{M(cal)}$	$t_{M(exp)}$	$t_{R(TPGC)}$	$t_{R(Teq)}$	%Error
18:1 ($n-9$)	165.11	1.831	1.828	8.620	8.611	0.10
18:2 ($n-6$)	166.26	1.832	1.853	9.703	9.715	-0.12
18:3 ($n-3$)	167.75	1.835	1.847	11.144	11.145	-0.01
20:1 ($n-9$)	169.18	1.837	1.841	12.463	12.494	-0.25
20:2 ($n-6$)	170.58	1.839	1.850	13.870	13.897	-0.19
22:1 ($n-9$)	174.81	1.845	1.863	16.806	16.869	-0.37
24:1 ($n-9$)	182.04	1.856	1.876	20.647	20.607	0.19
Four step (D)						
FAMES*	T_{eq}	$t_{M(cal)}$	$t_{M(exp)}$	$t_{R(TPGC)}$	$t_{R(Teq)}$	%Error
18:1 ($n-9$)	164.95	1.829	1.837	8.685	8.689	-0.05
18:2 ($n-6$)	165.95	1.831	1.838	9.785	9.739	0.47
18:3 ($n-3$)	167.62	1.833	1.847	11.196	11.145	0.46
20:1 ($n-9$)	169.09	1.836	1.841	12.502	12.494	0.06
20:2 ($n-6$)	170.75	1.838	1.849	13.803	13.788	0.11
22:1 ($n-9$)	174.96	1.844	1.870	16.685	16.623	0.37
24:1 ($n-9$)	182.49	1.856	1.886	20.251	20.242	0.04

* See the second footnote (*) in Table II.

Table IV. Comparison of ECL of Unsaturated FAMES of Chinese Mustard Seed Oil Calculated by Different Methods

FAMES	One step (A)			Two step (B)			Three step (C)			Four step (D)		
	ECL _(TPGC)	ECL _(Teq)	ECL _(G)	ECL _(TPGC)	ECL _(Teq)	ECL _(G)	ECL _(TPGC)	ECL _(Teq)	ECL _(G)	ECL _(TPGC)	ECL _(Teq)	ECL _(G)
18:1 (<i>n</i> –9)	18.36	18.35	18.35	18.34	18.35	18.35	18.34	18.35	18.35	18.35	18.35	18.35
18:2 (<i>n</i> –6)	18.97	18.96	18.96	18.95	18.96	18.96	18.96	18.96	18.96	18.95	18.96	18.96
18:3 (<i>n</i> –3)	19.72	19.70	19.70	19.69	19.70	19.70	19.70	19.70	19.70	19.70	19.70	19.70
20:1 (<i>n</i> –9)	20.35	20.34	20.35	20.32	20.34	20.33	20.33	20.34	20.33	20.33	20.34	20.33
20:2 (<i>n</i> –6)	20.96	20.95	20.95	20.94	20.95	20.95	20.94	20.95	20.95	20.95	20.95	20.94
22:1 (<i>n</i> –9)	22.34	22.33	22.33	22.31	22.33	22.33	22.32	22.33	22.32	22.32	22.33	22.32
24:1 (<i>n</i> –9)	24.33	24.29	NA*	24.29	24.29	NA	24.32	24.30	NA	24.33	24.30	NA

* NA: not analyzed.

Table V. Comparison of ECL Calculated by Different Methods

One step (A)					
FAMES	$t_{R(TPGC)}$	T_{eq}	$t_{M(cal)}$	$Z(TPGC)$	$Z(Teq)$
18:1	6.249	168.62	1.856	18.16	18.16
18:2	6.799	170.93	1.859	18.60	18.61
18:3	7.633	171.41	1.860	19.23	19.23
20:1	8.915	172.85	1.862	20.13	20.14
22:1	12.002	178.77	1.869	22.15	22.12
24:1	15.188	187.12	1.880	24.20	24.11
Two step (B)					
FAMES	$t_{R(TPGC)}$	T_{eq}	$t_{M(cal)}$	$Z(TPGC)$	$Z(Teq)$
C18:1	6.508	167.06	1.853	18.17	18.16
C18:2	7.13	169.12	1.856	18.61	18.61
C18:3	8.094	169.50	1.856	19.24	19.22
C20:1	9.625	170.85	1.858	20.14	20.13
C22:1	13.321	176.29	1.865	22.14	22.11
C24:1	16.461	185.26	1.877	24.18	24.10
Three step (C)					
FAMES	$t_{R(TPGC)}$	T_{eq}	$t_{M(cal)}$	$Z(TPGC)$	$Z(Teq)$
C18:1	6.433	166.91	1.835	18.16	18.16
C18:2	7.051	169.03	1.838	18.60	18.61
C18:3	8.012	169.43	1.838	19.22	19.22
C20:1	9.498	170.85	1.840	20.12	20.13
C22:1	12.772	176.95	1.848	22.13	22.11
C24:1	15.649	186.13	1.860	24.18	24.10
Four step (D)					
FAMES	$t_{R(TPGC)}$	T_{eq}	$t_{M(cal)}$	$Z(TPGC)$	$Z(Teq)$
C18:1	7.093	164.77	1.792	18.15	18.15
C18:2	7.654	167.65	1.796	18.59	18.60
C18:3	8.51	168.13	1.797	19.23	19.22
C20:1	9.873	169.84	1.799	20.15	20.13
C22:1	13.132	176.17	1.807	22.18	22.11
C24:1	16.177	185.27	1.819	24.23	24.10

Conclusion

Equation 12 is proposed for the determination of T_{eq} , which is used as the reference temperature for the correlation of the isothermal and TPGC data. According to Sun et al. (15), there are two different T_{eq} values in the literature. The first T_{eq} is the temperature at which the isothermal t_R is equal to $t_{R(TPGC)}$. The second T_{eq} is the temperature at which the isothermal I is equal to I_{TPGC} . However, the T_{eq} used in this study is the temperature at which the isothermal t_R is equal to $t_{R(TPGC)}$ and the isothermal ECL (or isothermal I) is equal to $ECL_{(TPGC)}$ (or I_{TPGC}).

The proposed method will allow analysts to make use of the large database of isothermal ECL or Kovats' retention index values. The I_{TPGC} database may not be necessary. The fluid dynamic parameter of the TPGC conditions is incorporated into equation 17 and the $I_{(Teq)}$ or $ECL_{(Teq)}$ is purely based on thermodynamic property of the solute. This will improve reproducibility of the retention data in TPGC among laboratories and may facilitate identification in TPGC.

Acknowledgments

This work was supported by the Thailand Research Fund.

References

1. E. Kovats. Gas chromatographic characterization of organic compounds. Part 1. Retention indexes of aliphatic halides, alcohols, aldehydes and ketones. *Helv. Chim. Acta.* **41**: 1915–32 (1958).
2. F.P. Woodford and C.M. van Gent. Gas-liquid chromatography of fatty acid methyl esters: the "carbon-number" as a parameter for comparison of columns. *J. Lipid Res.* **1**: 188–90 (1960).
3. T.K. Miwa, K.L. Micolajczak, F.R. Earle, and I.A. Wolff. Gas chromatographic characterization of fatty acids: Identification constants for mono- and dicarboxylic methyl esters. *Anal. Chem.* **32**: 1739–42 (1960).
4. K. Kittiratanapaiboon, N. Jeyashoke, and K. Krisnangkura. The relationship of Kovats retention indices and equivalent chain lengths of fatty acid methyl esters on a methyl silicone capillary column. *J. Chromatogr. Sci.* **36**: 361–64 (1998).

5. R. Lebrón-Aguilar, J.E. Quintanilla-Lopez, and J.A. García Domínguez. Improving the accuracy of Kovats retention indices in isothermal gas chromatography. *J. Chromatogr. A* **945**: 185–94 (2002).
6. V. Pacakova and L. Pelt. Chromatographic Retention Indices-An Aid to Identification of Organic Compounds. Ellis Harwood, New York, NY, 1992, pp. 64–79.
7. E. Tudor. Analysis of the equations for the temperature dependent of the retention index: I Relation between equations. *J. Chromatogr. A* **858**: 65–78 (1999).
8. H. Van den Dool and P.D. Kratz. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J. Chromatogr.* **11**: 463–71 (1963).
9. I.G. Zenkevich and B.V. Ioffe. System of retention indices for a linear temperature programming regime. *J. Chromatogr.* **439**: 185–94 (1988).
10. W.A. Halang, R. Langlais, and E. Kugler. Cubic spline interpolation for the calculation of retention indices in temperature-programmed gas-liquid chromatography. *Anal. Chem.* **50**: 1829–32 (1987).
11. T.S. Wang and Y.L. Sun. Definitions and methods of calculation of the temperature-programmed retention index, ITP: I The relationship between ITP and the net retention volume for n-alkanes. *J. Chromatogr.* **390**: 261–67 (1987).
12. L. Podmaniczsky, L. Szepeszy, K. Lakszner, and G. Sehomburg. Determination of retention indexes in LPTGC. Part IV. *Chromatographia* **21**: 387–91 (1986).
13. A. Zhu. Calculation of retention indices in temperature-programmed capillary gas chromatography. *J. Chromatogr.* **331**: 229–35 (1985).
14. B.J. Chen, X.J. Guo, and S.Y. Peng. Calculation of retention indexes in temperature programmed gas chromatography. *Chromatographia* **23**: 888–92 (1987).
15. Y. Sun, R. Zhang, Q. Wang, and B. Xu. Programmed temperature gas chromatographic retention index. *J. Chromatogr. A* **657**: 1–15 (1993).
16. F.R. González and A.M. Nardillo. Retention index in temperature-programmed gas chromatography. *J. Chromatogr. A* **842**: 29–49 (1999).
17. G. Guiochon. Retention indices in programmed temperature gas chromatography. *Anal. Chem.* **36**: 661–63 (1964).
18. J.C. Giddings. Gas Chromatography. N. Brenner, J. E. Callen, and M.D. Weiss, Eds. Academic Press, New York, NY, 1962, pp. 57–77.
19. H.W. Habgood and W.E. Harris. Retention indices in programmed temperature gas chromatography. *Anal. Chem.* **36**: 663–65 (1964).
20. J. Lee and D.R. Taylor. Relationship between temperature programmed and isothermal Kovats retention indexes in gas-liquid chromatography. *Chromatographia* **16**: 286–89 (1982).
21. J. Curvers, J. Rijks, C. Cramers, K. Knauss, and P. Larson. Temperature programmed retention indices: calculation from isothermal data part 1. *J. High Resolut. Chromatogr. Chromatogr. Commun.* **8**: 607–10 (1985).
22. S. Dal Nogare and W.E. Langlois. Programmed temperature gas chromatography. *Anal. Chem.* **32**: 767–70 (1960).
23. J. Giddings. Retention times in programmed temperature gas chromatography. *J. Chromatogr.* **4**: 11–20 (1960).
24. E.E. Akphorhonor, S. Le Vent, and D.R. Taylor. Calculation of programmed temperature gas chromatography characteristics from isothermal data: Theory and computational procedures. *J. Chromatogr.* **405**: 67–76 (1987).
25. Y.F. Guan, J. Kiraly, and J. Rijks. Interaction retention index database for compound identification in temperature-programmed capillary gas chromatography. *J. Chromatogr.* **472**: 129–43 (1989).
26. J. Chen, L. Zhang, Y. Tian, and L. Wang. Novel approach for the prediction of retention times in operating parameter programmed gas-liquid chromatography with capillary columns. *J. Chromatogr. A* **795**: 305–17 (1998).
27. N.H. Snow and H.M. McNair. A numerical simulation of temperature-programmed gas chromatography. *J. Chromatogr. Sci.* **30**: 271–75 (1992).
28. E.J. Cavalli and C. Guinchard. Forecasting retention times in temperature-programmed gas chromatography. *J. Chromatogr. Sci.* **33**: 370–76 (1995).
29. E.J. Cavalli and C. Guinchard. Forecasting retention times in temperature-programmed gas chromatography: experimental verification of the hypothesis on compound behavior. *J. Chromatogr. Sci.* **34**: 547–49 (1996).
30. K. Kittiratanapaiboon, N. Jeyashoke, and K. Krisnangkura. Forecasting retention times of fatty acid methyl esters in temperature-programmed gas chromatography. *J. Chromatogr. Sci.* **36**: 541–46 (1998).
31. I. Amasaki, Z. Gao, T. Kaneko, and M. Nakada. Calculation of retention times for temperature-programmed gas chromatography. *Anal. Sci.* **16**: 1229–31 (2000).
32. A.J.P. Martin. Some theoretical aspects of partition chromatography. *Biochem. Soc. Symp. (Partition Chromatography)* **3**: 4–20 (1950).
33. E. Tudor and T. Oncescu. Influence of temperature and n-alkane pair on the methylene increments to the thermodynamic functions of solution on SE-30 and Carbowax-20M capillary columns. *J. Chromatogr. A* **844**: 201–15 (1999).
34. F.R. González. Interpreting the gas chromatographic retention of n-alkanes. *J. Chromatogr. A* **873**: 209–19 (2000).
35. P. Kalayasiri, N. Jeyashoke, and K. Krisnangkura. Survey of seed oils for use as diesel fuels. *J. Am. Oil Chem. Soc.* **73**: 471–74 (1996).
36. K. Krisnangkura, A. Tancharoon, C. Konkao, and N. Jeyashoke. An alternative method for determination of equivalent chain length or carbon number of fatty acid methyl esters in gas chromatography. *J. Chromatogr. Sci.* **35**: 329–32 (1997).
37. T. Watanachaiyong, N. Jeyashoke, and K. Krisnangkura. A convenient method for routine estimation of dead time in gas chromatography. *J. Chromatogr. Sci.* **38**: 67–71 (2000).
38. S. Lomsugarit, J. Katsuwon, N. Jeyashoke, and K. Krisnangkura. An empirical approach for estimating equivalent chain length of fatty acid methyl ester in multistep temperature-programmed gas chromatography. *J. Chromatogr. Sci.* **39**: 468–72 (2001).
39. B. Koppenhoefer, G. Laupp, and M. Hummel. Temperature dependence of dead time as determined by methane retention in open-tubular capillary gas chromatography. *J. Chromatogr.* **547**: 239–45 (1991).