# An Alternative Method for the Calculation of Equivalent Chain Length or Carbon Number of Fatty Acid Methyl Esters in Gas Chromatography

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## Abstract

**The carbon number or equivalent carbon number (ECN) of fatty acid methyl esters (FAMEs) separated on a 30-m × 0.32-mm-i.d. Omegawax 320 capillary column is calculated directly from the retention data. When the described equation is used to identify FAMEs in a mixture of FAMEs from rambutan and para-rubber seed oils, the calculated ECN values are very close to the widely accepted equivalent chain length values described in the literature. Hence, the equation can probably be used as an identification tool for FAMEs.**

## Introduction

Gas chromatographic (GC) identification of fatty acid methyl esters (FAMEs) can be performed by several methods (1–5). The equivalent chain length (ECL) and equivalent carbon number (ECN) methods proposed by Miwa et al. (4) and Woodford and van Gent (5) are widely accepted today for identification of FAMEs. At least two or three normal saturated FAMEs are required as references, and other fatty acids including unsaturated fatty acids are characterized by their ECL. It is often advantageous to use the modified ECL in the identification of unsaturated FAMEs (6). The ECL of polyunsaturated FAMEs could be calculated from the equation

$$ECL_x = a_x ECL_{(18:3\omega3)} + b_x \qquad \text{Eq 1}$$

where $a_x$ and $b_x$ are constants and $ECL_{(18:3\omega3)}$ is the ECL of alpha linolenic acid methyl ester. ECL could also be obtained from the tabulated ECL values compiled by Jamieson (7,8).

Attempts have also been made to develop novel methods of GC identification. Gerbino and Castello (9) correlated the logarithms of retention times to those of the vapor pressure of halogenated hydrocarbons and used their vapor pressure for identification. Guillaume and Guinchard (10) developed a very useful equation for the prediction of capacity ratios for esters of *p*-hydroxy benzoic acids at various isothermal temperatures by using their connectivity index.

In this paper, we extended the equation of Vigh and Varga-Puchony (11) to calculate the ECL of FAMEs on Omegawax 320 capillary columns by using the ECN. The calculated ECN were very close to the ECL, but the methods of calculation were very different from that of Miwa et al. (4).

## Experimental

### Materials

Fatty acids of various chain lengths were purchased from Sigma Chemical (St. Louis, MO). Rambutan (*Naphelium lappaceum*) and para-rubber seeds (*Hevea brasiliensis*) were obtained from a field. Methyl esters of plant seed oils were prepared by acid-catalyzed transesterification in situ as described by Harrington and D'arcy-Evans (12) with a slight modification. Seed kernels were ground in a mortar. About 20 mg of the ground sample was transferred to a 20-mL screw-capped tube. Then 5 mL of 4% (v/v) methanolic $H_2SO_4$ was added, and the tube was capped tightly with a polytetrafluoroethylene liner cap. The reaction tube was kept at 70°C in a water bath for 5 h and cooled to room temperature by running tap water. Hexane and water (5 mL each) were added to the reaction tube. The hexane layer was washed twice with 2 mL of distilled water each time and dried over anhydrous sulfate. The obtained FAMEs were used without further distillation.

### GC

GC analysis was performed on a Shimadzu model 14A GC equipped with a flame-ionization detector, split–splitless injector, and a C-R4A data processor (Shimadzu, Kyoto, Japan). An Omegawax 320 (30 m × 0.32-mm i.d.; film thickness, 0.25 µm) capillary column (Supelco, Bellefonte, PA) was used with a nitrogen carrier gas flow rate of 1–2 mL/min. Oven temperatures were held isothermally at intervals between 170 and 200°C.

### Theory

The Vigh and Varga-Puchony equation (11) for prediction of the retention times of many homologues

$$\ln \frac{t_R - t_0}{t_0} = a + bn + \frac{c}{T} + \frac{dn}{T} \qquad \text{Eq 2}$$

can also be derived from the temperature dependent of retention:

$$\ln k' = -\frac{\Delta\mu^0}{RT} + \ln \beta \qquad \text{Eq 3}$$

where $k'$ is the capacity ratio of the solute:

$$k' = -\frac{t_R - t_0}{t_0} \qquad \text{Eq 4}$$

where $t_R$ is the retention time of the FAME; $t_0$ is the retention time of an unretained compound; $R$ is the gas constant; $T$ is the absolute temperature; $a$, $b$, $c$, and $d$ are constants; $n$ is the carbon number for the saturated compound, and $\beta$ is the phase ratio of the column.

$\Delta\mu^0$ is the standard chemical potential difference and linearly relates to standard molar enthalpy ($\Delta H^0$) and standard molar entropy ($\Delta S^0$) as follows:

$$\Delta\mu^0 = \Delta H^0 - T\Delta S^0 \qquad \text{Eq 5}$$

For normal paraffin, the following relationship has been established:

$$\Delta\mu_n{}^0 = \Delta\mu^0 - n\delta\mu \qquad \text{Eq 6}$$

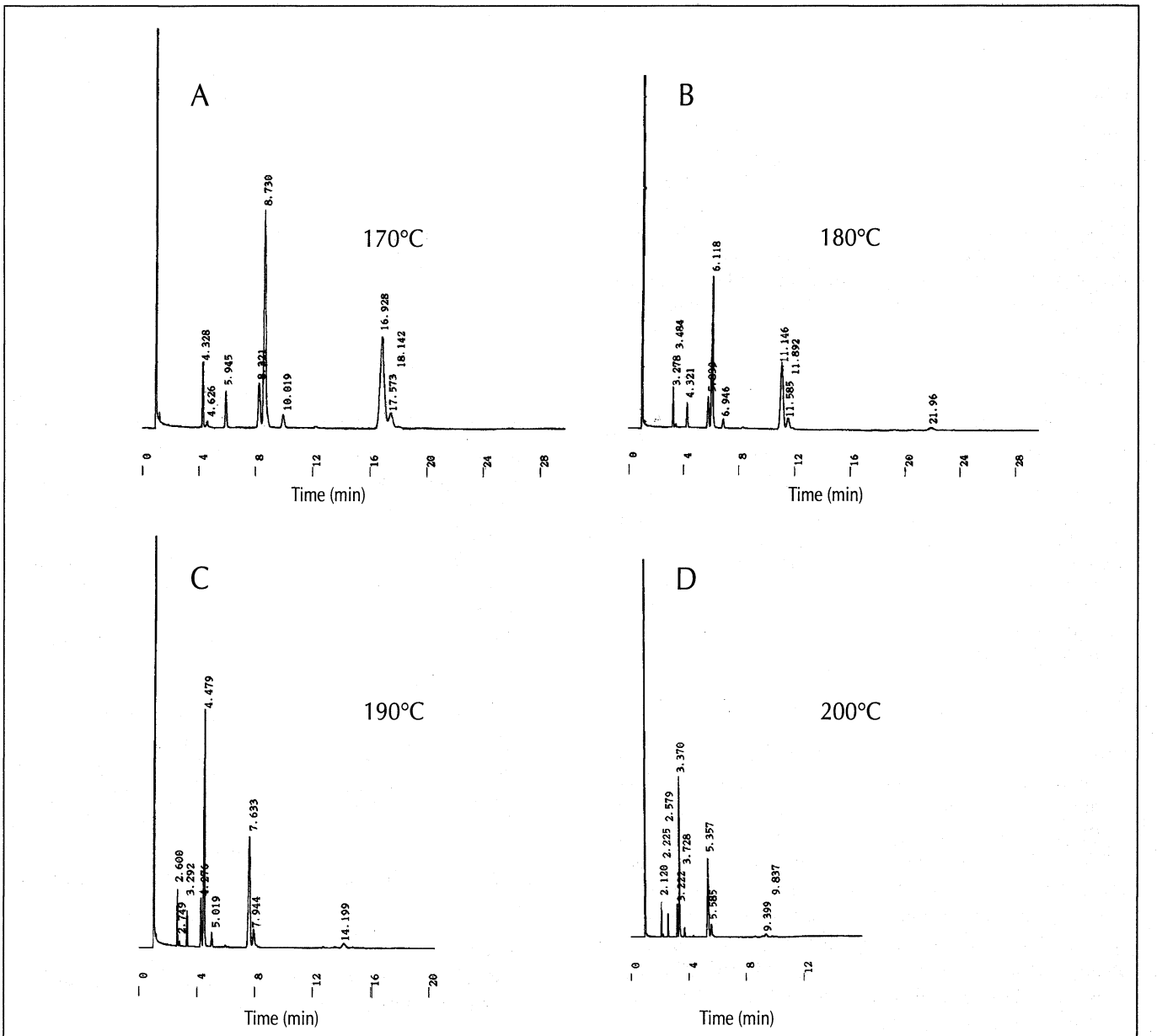


**Figure 1.** Gas chromatogram of FAMEs from a mixture of para-rubber and rambutan seed oil at various temperatures. Numbers on top of peaks are retention times (in minutes) of the peaks printed out by the C-R4A data processor.

where $\Delta\mu_n$ is the chemical potential of hydrocarbon of $n$ carbon atoms and $\delta\mu$ is the increment. Substituting Equations 5 and 6 into Equation 3 will result in the following:

$$\ln k' = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} - \frac{n\delta H}{R} + \frac{n\delta S}{R} + \ln \beta \qquad \text{Eq 7}$$

or

$$\ln \frac{t_R - t_0}{t_0} = a + bn + \frac{c}{T} + \frac{dn}{T} \qquad \text{Eq 8}$$

where

$$a = \frac{\Delta S^0}{R} + \ln \beta,\ b = \frac{\delta S}{R},\ c = -\frac{\Delta H^0}{R},\ \text{and}\ d = -\frac{\delta H}{RT}$$

## Results and Discussion

### Determination of constants

At a constant absolute temperature ($T$), Equation 8 is reduced to:

$$\ln \frac{t_R - t_0}{t_0} = a' + b'n \qquad \text{Eq 9}$$

where

$$a' = a + \frac{c}{T} \qquad \text{Eq 10}$$

and

$$b' = b + \frac{d}{T} \qquad \text{Eq 11}$$

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 $b$.

Alternatively, if $n$ is held constant, Equation 8 is reduced to:

$$\ln \frac{t_R - t_0}{t_0} = a'' + \frac{b''}{T} \qquad \text{Eq 12}$$

where

$$a'' = a + bn \qquad \text{Eq 13}$$

and

$$b'' = c + dn \qquad \text{Eq 14}$$

Thus, plotting $a''$ against $n$ would result in a straight line with a slope of $b$ and an intercept of $a$. Plotting $b''$ against $n$ would also give a straight line with a slope of $d$ and an intercept of $c$. Figure 1 shows the chromatogram of FAMEs with 16–22 carbon

**Table I. Logarithms of FAME Capacity Ratios on the Omegawax 320 Capillary Column at Temperatures between 170 and 200°C**

| | $1/T \times 10^3$ | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Carbon number | 2.114 | 2.137 | 2.160 | 2.183 | 2.208 | 2.232 | 2.257 | Intercept ($a''$) | Slope ($b''$) |
| 16 | 0.171 | 0.367 | 0.507 | 0.702 | 0.890 | 1.099 | 1.271 | –16.143 | 7.718 |
| 17 | 0.502 | 0.675 | 0.852 | 1.060 | 1.248 | 1.470 | 1.662 | –16.836 | 8.196 |
| 18 | 0.832 | 1.042 | 1.198 | 1.422 | 1.609 | 1.842 | 2.045 | –17.079 | 8.472 |
| 19 | 1.162 | 1.349 | 1.543 | 1.767 | 1.970 | 2.215 | 2.426 | –17.717 | 8.924 |
| 20 | 1.493 | 1.716 | 1.888 | 2.095 | 2.328 | 2.587 | 2.819 | –18.053 | 9.241 |
| 22 | 2.155 | 2.362 | 2.579 | 2.814 | 3.046 | 3.330 | 3.596 | –19.199 | 10.089 |
| Intercept ($a'$) | –5.118 | –4.993 | –5.019 | –4.912 | –4.862 | –4.852 | –4.922 | –8.296 ($a$) | –0.493 ($b$) |
| Slope ($b'$) | 0.331 | 0.335 | 0.345 | 0.351 | 0.359 | 0.372 | 0.387 | 1530 ($c$) | 388 ($d$) |

**Table II. ECNs of Some FAMEs Calculated with Equation 15 and Their ECL**

| | 170°C | | 180°C | | 190°C | | 200°C | | | ECL* |
|---|---|---|---|---|---|---|---|---|---|---|
| Solutes | $t_R$ (min) | ECN | $t_R$ (min) | ECN | $t_R$ (min) | ECN | $t_R$ (min) | ECN | Average | (Carbowax) |
| Hexane | 0.95 | – | 0.955 | – | 0.97 | – | 0.98 | – | – | – |
| Methyl palmitate | 4.328 | 15.96 | 3.278 | 15.98 | 2.6 | 15.97 | 2.12 | 15.94 | 15.96 | – |
| Methyl palmitoleate | 4.626 | 16.18 | 3.484 | 16.21 | 2.749 | 16.22 | 2.225 | 16.20 | 16.20 | 16.18 |
| Methyl stearate | 8.321 | 18.00 | 5.833 | 18.02 | 4.276 | 18.02 | 3.222 | 18.00 | 18.01 | – |
| Methyl oleate | 8.73 | 18.14 | 6.118 | 18.17 | 4.479 | 18.19 | 3.37 | 18.20 | 18.18 | 18.16 |
| Methyl linoleate | 10.009 | 18.54 | 6.946 | 18.58 | 5.019 | 18.60 | 3.728 | 18.63 | 18.59 | 18.58 |
| Methyl α-linolenate | 12.614 | 19.20 | 8.392 | 19.18 | 5.952 | 19.21 | 4.336 | 19.24 | 19.21 | 19.18 |
| Methyl eicosanoate | 16.928 | 20.02 | 11.14 | 20.04 | 7.633 | 20.05 | 6.357 | 20.00 | 20.03 | – |
| Methyl 9-eicosenoate | 17.529 | 20.12 | 11.585 | 20.16 | 7.944 | 20.18 | 5.585 | 20.19 | 20.16 | 20.14 |
| Methyl docosanoate | 35.219 | 22.02 | 21.962 | 22.05 | 14.195 | 22.04 | 9.399 | 22.05 | 22.04 | – |

* Values obtained from Christie (13).

atoms analyzed isothermally at temperatures between 170 and 200°C. The $t_0$ and capacity ratios are reported in Table I. The slopes, intercepts, and the four constants (*a, b, c,* and *d*) were analyzed on a personal microcomputer with Microsoft Excel version 5.0. The results are summarized in Table I. Rewriting Equation 8 with the numeric values of the four constants resulted in the following:

$$\ln \frac{t_R - t_0}{t_0} = -8.296 - 0.493n + \frac{1530}{T} + 388\frac{n}{T} \qquad \text{Eq 15}$$

Equation 15 can be used to estimate the ECNs of both saturated and unsaturated FAMEs. It can also be used to predict the absolute retention times of FAMEs with 16–22 carbon atoms at temperatures between 170 and 200°C. Retention times of FAMEs with carbon atoms outside this range can be predicted, but probably with less accuracy. Similarly, reduced accuracy would be expected for analysis outside the range of the above temperatures.

**Estimation of some common FAMEs at various temperatures**

Table II summarizes the ECNs of FAMEs with 16–22 carbon atoms commonly found in rambutan and para-rubber seed oils. The ECL of these FAMEs on a Carbowax 20M column reported by Christie (13) has also been included for comparison. The calculated ECNs of the saturated FAMEs were very close to their actual values at all temperatures between 170 and 200°C. The calculated ECNs of mono-unsaturated FAMEs (16:1, 18:1, and 20:1) were 16.20, 18.18, and 20.16, respectively. The ECNs of these FAMEs were very close to the ECL values reported by Christie (last column). The calculated ECNs of polyunsaturated FAMEs (18:2 and α-18:3) were also close to the reported values.

## Conclusion

The proposed equation could provide a very convenient method for the calculation of ECN and could be used as a tool for the identification of FAMEs. The ECN can be calculated without any reference. It can also be used to estimate the capacity ratio of FAMEs from their ECL. When $t_0$ is known, the absolute retention time can then be accurately predicted. Also, it is expected that the above equation would be able to cover polyunsaturated FAMEs as well, and the tabulated ECL of polyunsaturated FAMEs reported by Jamieson (7,8) would extend the usefulness of the proposed equation. At the present time, however, the proposed equation can be used to calculate the ECN between 16 and 22 carbon atoms and at temperatures between 170 and 200°C. For FAMEs with shorter or longer chain lengths, validity of the equation has not been tested. It has been noted that a straight line relationship between the logarithms of retention times and the number of carbon atoms may not hold (6,13). Also, it is expected that, for operating temperatures outside the above range, the accuracy of the calculated ECN may be reduced. Another disadvantage of the proposed equation is that the four constants may change as the diameter of the column or the thickness of the liquid phase is changed. Also, it is expected that the four constants may change as the column ages. This is partly due to the change in chemical structure of the liquid phase by oxidation or thermal hydrolysis, which may change the entropy and enthalpy of the solution.

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