Effect of Column Length on Forecasted Retention Times and Carbon Numbers in an Isothermal and Temperature-programmed Gas Chromatography

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Received 23 February 1999

ABSTRACT The retention factor (k') of a fatty acid methyl ester (FAME) in a 30 meter Omegawax 250 capillary column can be accurately predicted by the following equation:

$$\ln k' = -7.55 - 0.48n + \frac{1496}{T} + \frac{379n}{T}$$

Where n is the carbon number or equivalent carbon number (ECN) and T is the absolute temperature. The four numeric constants of the above equation are not sensitive to column length and they can be employed to calculate the ECN of FAMEs eluted out from the shorter columns of 27, 24 and 21 meters. In addition, these four numeric constants can be employed to forecast the retention times of FAMEs eluted out from the aforementioned columns in the temperature-programmed gas chromatography (TPGC).

KEYWORDS: Column length, column constants, retention time, temperature-programmed gas chromatography.

INTRODUCTION

Gas chromatographic (GC) identification of organic compounds depends very much on their retention behaviors in a given column. The retention index system proposed by Kovats in 1958 has been widely accepted as an identification aid for organic compound[1] because it is considered to express the retention with the best reproducibility and precision. Latter, similar retention systems were proposed for identification of specific functional groups of compounds by using other references. Among these are equivalent chain length (ECL)[2] and carbon number (n)[3] which have been employed for the identification of fatty acid methyl esters (FAMEs). The major disadvantage of the original method of calculation of retention index and ECL is the requirement of at least two reference standards in all identification.

Recently, it has been demonstrated that the retention factors (k') of FAMEs relate to their carbon number or ECL by the following equation (Eq 1)[4]:

$$\ln k' = a + bn + \frac{c}{T} + \frac{dn}{T} \qquad \text{Eq 1}$$

where T is the absolute temperature and a, b, c and d are column constants which relate to thermodynamic parameters as follows:

$$a = \frac{\Delta S^0}{R} + \ln\beta \qquad \text{Eq 2}$$

$$b = \frac{\delta S}{R} \qquad \text{Eq 3}$$

$$c = -\frac{\Delta H^0}{R} \qquad \text{Eq 4}$$

$$d = -\frac{\delta H}{R} \qquad \text{Eq 5}$$

where β is phase ratio of the column. ΔH^0 and ΔS^0 are standard molar enthalpy and entropy, respectively. ΔH^0 and ΔS^0 are the increments in enthalpy and entropy, respectively, with respect to carbon number. Eq 1 would be an alternative method of calculation of ECL without a reference standard. Also, the ECL of a FAME can be converted to retention index or vice versa by the same equation.[5] Furthermore, combining Eq 1 with that of Calvalli and Guinchard[6] on column slicing method, a new equation was obtained to forecast the retention times of FAMEs in the temperature-programmed GC.[7] Thus, Eq 1 may probably revolutionize the gas chromatographic identification of organic compounds without a reference standard. The correctness in identification of organic compounds or forecasting

their retention times by Eq 1 depends very much on the four column constants, a, b, c and d. Any factor which affect the change in one of these column constant is undoubtedly would affect the accuracy of the identification. Theoretically, it may be speculated that column length should not affect the four constants, but there has been no experimental support for this.

In this paper, we present experimental data to support the theoretical assumption that column length has no effect on the values of the four parameters.

Experimental

Materials

Fatty acid methyl esters of various chain lengths were purchased from Sigma Chemical Co (St Louis, MO). Rambutan (*Naphelium lappaceum*) and para-rubber seed were obtained from the field. Transmethylation of plant seed oils were carried out in situ with acid catalysis as described by Kalayasiri *et al*.[8]

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). The same Omegawax 250 (30 m x 0.25-mm id; film thickness, 0.25 µm) capillary column reported by Kittiratanapaiboon *et al*[7] was used in this study. Column was cut short by 3 meters at a time to the final length of 21 meters. Nitrogen was used as a carrier gas at the flow rate 0.7-1.5 mL/min. Injector and detector temperature were set at 250°C

Results and Discussion

The four column constants, a, b, c and d reported by Kittiratanapaiboon *et al*[7] were -7.55, 0.48, 1496 and 379, respectively. Substitution these numeric values into Eq 1 give equation 6.

$$\ln k' = -7.55 - 0.48n + \frac{1496}{T} + \frac{379n}{T} \qquad \text{Eq 6}$$

The column has occasionally been employed for the analysis of FAMEs and the last determination of the four constants was over 6 months. Prior to the cutting of the column, a mixture of FAMEs of para-rubber and rambutan seed was injected at temperature between 170°C and 200°C to verify that the four numeric values in Eq 6 are still valid for this column. The calculated ECLs of FAMEs (not shown) were very closed to those reported earlier,[4] suggesting that the four numeric values are not appreciably changed and equation 6 is still valid for the calculation of the ECL of FAMEs. When the column was shorten 3 meters each to 27, 24, and 21 meters, the calculated ECLs of FAMEs at various temperatures were very close to those reported in literature as summarized in Table I to III, respectively. The calculated ECL of FAMEs at different column lengths did not appreciably deviate from those reported earlier.[4] The higher difference was observed for the unsaturated FAMEs with more double bond especially, at higher temperature. This was not unexpected due to temperature effect on the polarity of the stationary phase[9]

Temperature-programmed GC (TPGC)

Recently, it has been shown that the four column constants of Eq 1 can be employed to forecast the retention times of FAMEs in TPGC[7] by rearranging to Eq 7 and using the column slicing method of Calvalli and Guinchard.[6]

$$t_R = \sum_{i=1}^{m} \frac{t_0}{m}\left(1 + e^{(a + bn + \frac{c}{T_i} + \frac{dn}{T_i})}\right) \qquad \text{Eq 7}$$

where m is the element numbers and T_i is the temperature of the i elements. Fig 1 (A-B) is a non-linear temperature programmed gas chromatograms of FAMEs run from 170°C to 220°C at a temperature gradient of 4°C/min. Initial hold time for Fig 1 (A and B) is 1 minute and C is 1.5 minutes. The column lengths are 27(A), 24(B) and 21 meters (C), respectively. Retention times are printed at the peak tops. The predicted retention times were calculated according to Kittiratanapaiboon *et al*[7] and results were summarized in Table 4. The highest difference between the predicted and experimental values was about 1.5% for alpha methyl linolinate. The large difference for the methyl linolinate may be the ECL value (19.21) used for the calculation is too low as was shown in the isothermal GC (Table 1-3)

Results in this study support the theoretical assumption that the four thermodynamic parameters, (ΔH^0, ΔS^0, δH^o and δS^o) which arise from the interaction between the liquid phase and the solutes are not changed. Also, the phase ratio of the column, β, is not affected by column length. Thus, the four numeric constants should not be changed when the column was cut short. This finding would be beneficial and make the researcher at ease when the column is broken accidentally.

Table 1. Calculated equivalent carbon numbers of FAMEs eluted from an Omegawax 250 capillary column (27 meters) at temperature between 170 and 200°C.

		170 °C (t_0=0.618)		180 °C (t_0 =0.637)		190 °C (t_0 =0.635)		200 °C (t_0 =0.643)	
FAMEs	**ECL**	t_R	**ECN**	t_R	**ECN**	t_R	**ECN**	t_R	**ECN**
16:0	16.00	4.529	16.03	3.380	16.00	2.538	16.00	2.016	15.99
17:0	17.00	6.341	17.04	4.573	17.02	3.326	17.02	2.551	17.02
18:0	18.00	8.950	18.04	6.277	18.02	4.432	18.04	3.291	18.04
18:1	18.18	9.544	18.22	6.700	18.23	4.724	18.26	3.497	18.27
18:2	18.59	11.199	18.68	7.781	18.69	5.423	18.72	3.917	18.70
18:3	19.21	14.042	19.31	9.597	19.32	6.595	19.37	4.715	19.38
20:0	20.00	18.287	20.04	12.158	20.03	8.130	20.05	5.704	20.06
20:1	20.18	19.281	20.19	12.875	20.20	8.633	20.24	6.055	20.22

Table 2. Calculated equivalent carbon numbers of FAMEs eluted from an Omegawax 250 capillary column (24 meters) at temperature between 170 and 200°C.

		170 °C (t_0=1.096)		180 °C (t_0 =1.02)		190 °C (t_0 =1.106)		200 °C (t_0 =1.072)	
FAMEs	**ECL**	t_R	**ECN**	t_R	**ECN**	t_R	**ECN**	t_R	**ECN**
16:0	16.00	7.934	15.99	5.347	15.96	4.400	15.98	3.385	15.97
17:0	17.00	11.116	17.01	7.246	16.98	5.756	16.88	4.287	17.00
18:0	18.00	15.745	18.02	9.951	17.99	7.665	18.01	5.532	18.02
18:1	18.18	16.776	18.20	10.618	18.20	8.166	18.23	5.810	18.21
18:2	18.59	19.685	18.65	12.329	18.66	9.275	18.70	6.510	18.66
18:3	19.21	24.703	19.29	15.236	19.30	11.385	19.34	7.790	19.30
20:0	20.00	32.187	20.02	19.310	20.00	14.065	20.02	9.616	20.05
20:1	20.18	34.02	20.17	20.45	20.17	14.094	20.21	10.192	20.26

Table 3. Calculated equivalent carbon numbers of FAMEs eluted from an Omegawax 250 capillary column (21 meters) at temperature between 170 and 200°C.

		170°C (t_0=0.753)		180°C (t_0 =0.815)		190°C (t_0 =0.82)		200°C (t_0 =0.83)	
FAMEs	**ECL**	t_R	**ECN**	t_R	**ECN**	t_R	**ECN**	t_R	**ECN**
16:0	16.00	5.587	15.99	4.252	15.98	3.245	15.95	2.567	15.95
17:0	17.00	7.815	16.99	5.751	17.00	4.24	16.97	3.243	16.98
18:0	18.00	11.045	17.99	7.894	18.00	5.636	17.98	4.178	18.00
18:1	18.18	11.786	18.17	8.424	18.19	5.998	18.19	4.446	18.24
18:2	18.59	13.809	18.62	9.757	18.64	6.887	18.66	5.010	18.69
18:3	19.21	17.302	19.25	12.044	19.27	8.359	19.30	6.010	19.36
20:0	20.00	22.537	19.98	15.287	20.00	10.311	19.98	7.237	20.02
20:1	20.18	23.810	20.13	16.172	20.15	10.937	20.17	7.660	20.22

Table 4. Comparison of experimental and forecasted TPGC retention times of FAMEs eluted from capillary column of different length.

		27 meter (t_0=0.625)			24 meter (t_0=1.090)			21 meter (t_0=0.772)		
FAMEs	ECL[a]	Experimental t_r	Calculated t_r	%Δ[b]	Experimental t_r	Calculated t_r	%Δ	Experimental t_r	Calculated t_r	%Δ
16:0	16.00	3.923	3.96	-0.94	5.976	6.05	-1.24	4.828	4.842	-0.29
17:0	17.00	5.008	5.05	-0.84	7.354	7.43	-1.03	6.079	6.104	-0.41
18:0	18.00	6.285	6.33	-0.72	8.907	8.97	-0.71	7.509	7.548	-0.52
18:1	18.18	6.563	6.58	-0.26	9.251	9.26	-0.10	7.833	7.824	0.11
18:2	18.59	7.222	7.17	0.72	10.030	9.93	1.00	8.545	8.468	0.90
18:3	19.21	8.195	8.10	1.16	11.148	10.98	1.51	9.590	9.477	1.18
20:0	20.00	9.281	9.35	-0.74	12.33	12.33	0.00	10.760	10.805	-0.42
20:1	20.18	9.579	9.64	-0.64	12.688	12.65	0.30	11.073	11.113	-0.36

[a] from references 5 and 10

[b] %Δ = percent difference between calculated and experimental values

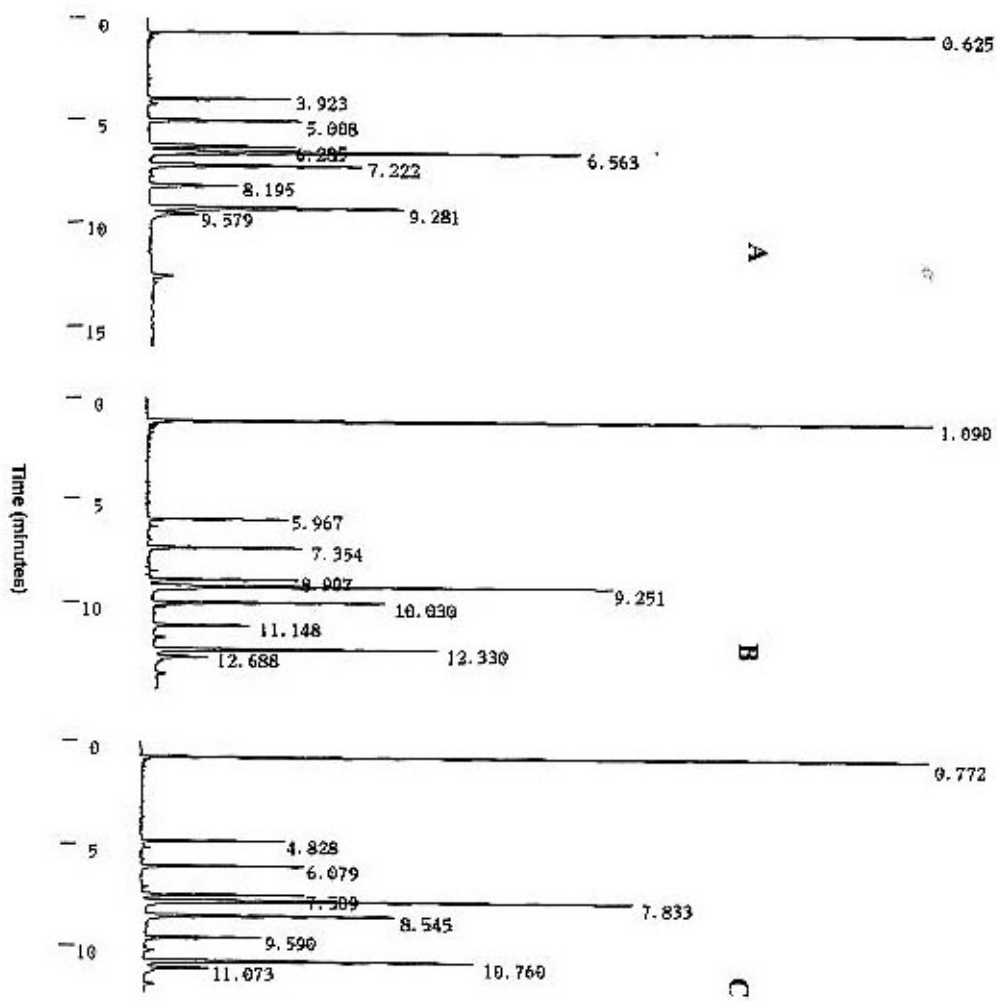

Fig 1. Gas chromatogram of FAMEs eluted from Omegawax 250 capillary columns (0.25 mm I.D.) from 170 °C to 220 °C with the temperature gradient of 4 °C/min. Final hold time was 60 min. Peak retention times are in minute(s).
A; column length 27 meters, initial hold time, 1 minute.
B; column length 24 meters, initial hold time, 1 minute.
C; column length 21 meters, initial hold time, 1.5 minutes.

Another interesting point which should be pointed out is that carrier gas flow rate does not very much affect the accuracy of the calculation. The carrier gas flow rates can be set as high as 3.5 mL/min and the calculated ECL of FAMEs were not deviate from those reported in Table 1-3. However, results were not included in this report because similar conclusion can be drawn from TPGC (Fig 1) which run at different carrier gas flow rate.

Conclusion

Experimental data collected at both isothermal and temperature programmed conditions can be concluded without any ambiguity that column lengths do not affect the numerical constants of Eq 6.

Acknowledgement

This work was supported by a grant from Thailand Research Fund.

References

1. Kováts E (1958) Gas chromatographic characterization of organic compounds. Part 1. Retention indexes of aliphatic halides, alcohols, aldehydes and ketones. *Helv Chim Acta* **41**, 1915-32.
2. Miwa TK *et al* (1960) Gas chromatographic characterization of fatty acids: Identification constants for mono- and di-carboxylic methyl esters. *Anal Chem* **32**, 1739-42.
3. Woodford FP and van Gent CM (1960) Gas-liquid chromatography of fatty acid methyl esters: The "carbon number" as a parameter for comparison of columns. *J Lipid Res* **1**, 188-90.
4. Krisnangkura K *et al* (1997) An alternative method for the calculation of equivalent chain length or carbon number of fatty acid methyl esters in gas chromatography. *J Chromatogr Sci* **35**, 329-32.
5. Kittiratanapaiboon K *et al* (1998) 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**, 361-4.
6. Cavalli EJ and Guinchard C (1996) Forecasting retention times in temperature-programmed gas chromatography: Experimental verification of the hypothesis on compound behavior. *J Chromatogr Sci* **34**, 547-9.
7. Kittiratanapaiboon K *et al* (1998) Forecasting retention Ttmes of fatty acid methyl esters in temperature-programmed gas chromatography. *J Chromatogr Sci* **36**, 541-6.
8. Kalayasiri P *et al* (1996) Survey of plants seed oil for use as diesel fuel. *J Am Oil Chem Soc* **73**, 471-4.
9. Castello G *et al* (1997) Effect of temperature on the polarity of some stationary phases for gas chromatography. *J. Chromatogr A* **779**, 275-86.