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USE OF EQUIVALENT CHAIN LENGTHS FOR THE CHARACTERIZATION OF FATTY ACID METHYL ESTERS SEPARATED BY LINEAR TEMPERATURE-PROGRAMMED GAS CHROMATOGRAPHY

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SUMMARY

C_{12}–C_{26} fatty acid methyl esters (FAMEs) were separated by glass capillary gas chromatography on SP 2340 as stationary phase, both isothermally in the temperature range 150–220°C and using linear temperature-programmed capillary gas chromatography (LTPCGC) with gradients of 0.5–3.5°C/min. The dependences of the equivalent chain lengths on temperature are linear. An equation as well as a graphic procedure are derived for the prediction of the retention temperatures of FAMEs separated by LTPCGC on SP 2340. Good agreement between predicted and experimental retention temperatures was found.

INTRODUCTION

Since 1959 when Lipsky *et al.*[1,2] demonstrated the use of capillary gas chromatography for the separation of fatty acid methyl esters (FAMEs), many papers have dealt with the application of this technique to the field of lipid research. Both non-polar (Apiezons and methylsilicones) and polar (various types of polyesters and polar silicones) liquids have been used as stationary phases. The highly polar siloxanes allow rapid and effective separation of FAMEs, including the separation of geometrical and positional isomers of unsaturated acids. Heckers *et al.*[3] demonstrated the potential of the cyanopropylsiloxane type of stationary liquid (SP 2340) in the glass capillary gas chromatographic separation of long chain FAMEs. Cyanoalkylpolysiloxanes (Silar 9CP, 10C, SP 2340, OV-275) have greater thermal stability than polyesters[4]. No significant variations in retention over a period of 6 months were found on Silar 5CP and 10C[5].

For tentative identification of fatty acids in lipids, relative retentions are used. The use of esters of normal saturated carboxylic acids as standards has found general acceptance in the analysis of FAMEs. The equivalent chain length (ECL) relationship

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was developed by Woodford and Van Gent[6] and Miwa *et al.*[7]

$$\mathrm{ECL} = z + \frac{\log (t'_{R,i}/t'_{R,z})}{\log (t'_{R,z+1}/t'_{R,z})} \quad (1)$$

where z is the number of carbon atoms in the alkyl chain of the normal saturated carboxylic acid and t'_R are corrected retention times which decrease in the order

$$t'_{R,z+1} > t'_{R,i} > t'_{R,z} \quad (2)$$

where i denotes the FAMEs of interest.

Golovnya *et al.*[5] preferred ECL values for identification purposes when a mixture consisted only of FAMEs, whereas Kovát's retention indices were used in the presence of other types of compounds, *e.g.*, long-chain hydrocarbons, alcohols, carbonyls and waxes. However, it should be stressed that ECL values can be used for tentative identification of FAMEs separated by gas chromatography only under isothermal conditions. We have shown that the dependence of ECL values on temperature, $\mathrm{d(ECL)/d}T$, can be used for tentative identification of the geometrical isomers of monounsaturated FAMEs[8]. In a narrow range of temperatures, the dependence of the ECL values can be considered linear and expressed by

$$\mathrm{ECL} = A + BT \quad (3)$$

where A and B are constants depending on the nature of the solute and chromatographic system. However, isothermal capillary gas chromatography of FAMEs is tedious in analytical practice or does not resolve all FAMEs, therefore the analyses are mostly performed under temperature-programmed conditions.

At present, the tentative identification of FAMEs separated by temperature-programmed gas chromatography (TPGC) cannot be performed using ECL values and therefore the available standards must be analyzed after each change in the temperature program. The aim of this paper is to describe a procedure for the tentative identification of FAMEs separated by linear temperature-programmed gas chromatography (LTPGC) using ECL values.

THEORETICAL

In linear temperature-programmed capillary gas chromatography (LTPCGC), we have used the following simplifying assumptions[9]:

(i) Linear temperature programming begins when the sample is injected. There is no isothermal period at the beginning of the analysis.

(ii) All compounds of interest are eluted under LTPCGC conditions.

(iii) The isothermal ECL values vary linearly with temperature [$\mathrm{d(ECL)/d}T$ = constant].

(iv) The experimental conditions, particularly the initial temperature and carrier gas flow-rate, are kept constant.

Under similar conditions, we have derived for hydrocarbons a formula for prediction of the retention temperature from isothermal Kovát's indices and elution temperatures of *n*-alkanes in LTPCGC[9].

Using this procedure for FAMEs chromatographed by TPCGC, we have derived the following equation for the retention temperature

$$T_{R,i} = \frac{(T_{R,z+1} - T_{R,z})[\text{ECL}\ (T_1) - T_1B - z] + T_{R,z}}{1 - B(T_{R,z+1} - T_{R,z})} \quad (4)$$

where T_R are the retention temperatures, i denoting the compound of interest, z is the number of carbon atoms in the alkyl chain of the normal saturated fatty acid methyl ester; ECL (T_1) is the value determined isothermally at temperature T_1 and B is the coefficient from eqn. 3.

EXPERIMENTAL

A Carlo Erba high resolution gas chromatograph, Model 4160, equipped with a flame ionization detector and a Spectra Physics integrator, Model SP-4000, were used for all analyses. The chromatograph was fitted with a glass capillary column (75 m × 0.3 mm I.D.) coated dynamically with cyanopropylsiloxane SP 2340 (Supelco, Bellefonte, PA, U.S.A.) as described elsewhere[10]. The analyses were performed isothermally at 150–220°C with a 10°C step, and using LTPGC from 150 to 230°C with gradients of 1–3°C/min and a step of 0.5°C. A Grob splitting system was incorporated in the instrument: the splitting ratio was 1:70. The carrier gas (hydrogen) flow-rate was 25–30 cm/s. The injector and detector temperatures were 235°C. The standards and a model mixture of fatty acid methyl esters were purchased from Supelco. The sample volumes injected were in the range of 1–5 μl of a 0.5% solution of the model mixture prepared from standards in hexane. The elution temperatures were obtained by multiplying the retention times by the temperature gradients and simultaneously read from the thermometer at the peak maxima.

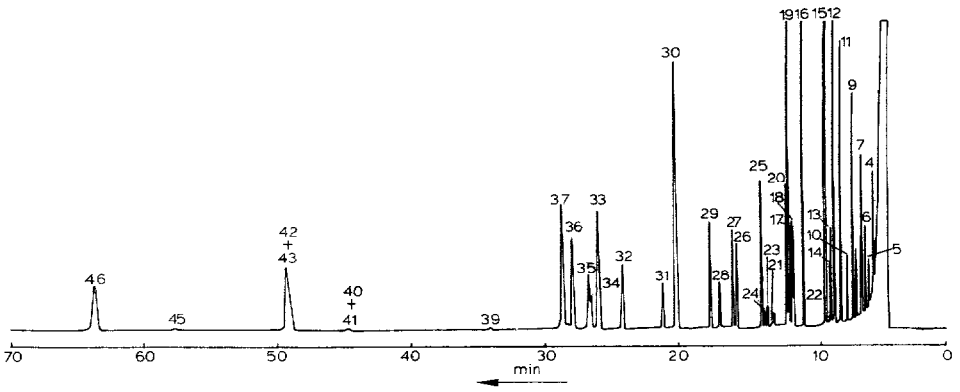

Fig. 1. Separation of FAMEs on a SP 2340 glass capillary column at 170°C. For other conditions, see Experimental. Peaks: 2 = 10:0; 4 = 12:0; 5 = 13:0; 6 = iso- 14:0; 7 = 14:0; 8 = aiso- 15:0; 9 = 15:0; 10 = iso- 16:0; 11 = 16:0; 12 = 16:1ω7t; 13 = 16:1ω7c; 14 = aiso- 17:0; 15 = 17:0; 16 = 18:0; 17 = 18:1ω9t; 18 = 18:1ω7t; 19 = 18:1ω9c; 20 = 18:1ω7c; 21 = 19:0; 22 = 18:2ω6t,t; 23 = 18:2ω6c,t; 24 = 18:2ω6t,c; 25 = 18:2ω6c,c; 26 = 18:3ω6c-all; 27 = 20:0; 29 = 20:1ω9c; 30 = 21:0; 31 = 20:2ω6c,c; 32 = 20:3ω6c-all; 33 = 22:0; 34 + 35 = 20:4ω6c-all + 20:3ω3c-all; 36 = 22:1ω9c; 39 = 20:5ω3c-all; 40 = 24:0; 41 = 22:4ω6c-all; 42 = 24:1ω9c; 43 = 22:4ω6c-all; 45 = 22:5ω6c-all; 46 = 22:6ω3c-all.

TABLE I

COEFFICIENTS OF THE EQUATION $\log t'_R = a + b/T$ FOUND USING THE METHOD OF LEAST SQUARES FOR FAMEs SEPARATED ON SP 2340 AT 150–220°C

r = Correlation coefficient.

FAME	*a*	*b*	*r*
9:0	−6.291	2637.8	0.9919
10:0	−5.658	2413.7	0.99986
11:0	−5.640	2460.6	0.99978
12:0	−5.797	2588.0	0.99901
13:0	−6.001	2738.2	0.99919
iso-14:0	−6.073	2794.9	0.9982
14:0	−6.150	2856.3	0.9990
aiso-15:0	−6.293	2961.2	0.9990
15:0	−6.340	298.5	0.9989
iso-16:0	−6.539	3116.2	0.9989
16:0	−6.600	3170.6	0.9992
16:1ω7t	−6.572	3184.6	0.9992
16:1ω7c	−6.496	3162.6	0.9989
aiso-17:0	−6.800	3300.3	0.9995
17:0	−6.829	3333.3	0.9993
18:0	−7.060	3490.4	0.9994
18:1ω9t	−6.979	3475.8	0.9994
18:1ω7t	−7.033	3502.6	0.9994
18:1ω9c	−6.936	3466.2	0.99949
18:1ω7c	−7.001	3498.9	0.99961
19:0	−7.302	3656.3	0.9996
18:2ω6tt	−7.434	3711.9	0.99968
18:2ω6c,t	−7.099	3572.7	0.99777
18:2ω6t,c	−7.035	3551.1	0.99956
18:2ω6c,c	−6.934	3512.1	0.99959
18:3ω6c-all	−6.918	3538.6	0.99961
20:0	−7.508	3805.2	0.99964
18:3ω3c-all	−6.998	3594.5	0.99960
20:1ω9c	−7.383	3776.4	0.99962
21:0	−7.802	3996.8	0.99962
20:2ω6c,c	−7.379	3819.7	0.99962
20:3ω6c-all	−7.307	3819.7	0.99970
22:0	−8.027	4154.5	0.99965
20:3ω3c-all	−7.429	3895.6	0.99977
20:4ω6c-all	−7.255	3818.2	0.99971
22:1ω9t	−7.919	4123.7	0.99968
22:1ω9c	−7.848	4098.4	0.99971
23:0	−8.208	4293.7	0.99969
20:5ω3c-all	−7.135	3815.3	0.99975
24:0	−8.296	4387.9	0.99977
22:4ω6c-all	−7.495	4033.9	0.99982
24:1ω9c	−8.117	4329.0	0.99981
25:0	−8.473	4522.8	0.99995
25:5ω3c-all	−7.555	4113.5	0.99979
22:6ω3c-all	−7.534	4125.4	0.99983
26:0	−8.675	4670.7	0.99996

RESULTS AND DISCUSSION

A model mixture of FAMEs was chromatographed isothermally in the region of 150–220°C on a glass capillary column coated with SP 2340. A typical chromatogram obtained at 170°C is shown in Fig. 1. From the obtained retention data, a linear dependence of log t'_R upon $1/T$ was found

$$\log t'_R = a + b/T \tag{5}$$

where t'_R is the corrected retention time, a and b are constants and, T is the column temperature. The coefficients of eqn. 5 were evaluated by the method of least squares. From the correlation coefficients, r, in Table I it is seen that eqn. 5 is valid for all FAMEs chromatographed at temperatures from 150 to 220°C. The slopes (coefficients b in eqn. 5) differ for all the FAMEs in Table I, from which it can be concluded that the problems with the separation of some pairs of FAMEs in Fig. 1 can be solved by temperature optimization in isothermal gas chromatography.

In Table II the coefficients of eqn. 3 are given, as determined using the method

TABLE II

COEFFICIENTS OF THE EQUATION ECL = $A + BT$ FOUND USING THE METHOD OF LEAST SQUARES FOR FAMEs SEPARATED ON SP 2340 AT 150–220°C

FAME	*A*	*B*	*r*
iso-14:0	13.40	0.0005861	0.2525
aiso-15:0	14.76	−0.0002664	−0.4292
iso-16:0	15.73	−0.001250	−0.7623
16:1ω7t	16.02	0.00260	0.9652
16:1ω7c	15.75	0.00532	0.9480
aiso-17:0	16.90	−0.00109	−0.6028
18:1ω9t	17.84	0.003114	0.9988
18:1ω7t	18.05	0.00220	0.9832
18:1ω9c	17.72	0.00470	0.9967
18:1ω7c	17.96	0.00376	0.9795
18:2ω6t,t	18.38	0.00300	–
18:2ω6c,t	18.01	0.00682	0.9931
18:2ω6t,c	18.15	0.00639	0.9930
18:2ω6c,c	17.85	0.00871	0.9985
18:3ω6c-all	17.94	0.01162	0.9983
18:3ω3c-all	18.26	0.01180	0.9986
20:1ω9c	19.55	0.00525	0.9950
20:2ω6c,c	19.66	0.00905	0.9970
20:3ω6c-all	19.60	0.01251	0.9980
20:3ω3c-all	20.13	0.01152	0.9974
20:4ω6c-all	19.63	0.01462	0.9982
22:1ω9t	21.78	0.00295	0.9945
22:1ω9c	21.60	0.00468	0.9975
22:5ω3c-all	19.94	0.01840	0.9779
22:4ω6c-all	21.04	0.01731	0.9980
24:1ω9c	23.44	0.00531	0.9806
22:5ω6c-all	21.7	0.01917	0.9964
22:5ω3c-all	21.60	0.01949	0.9878
22:6ω3c-all	21.6	0.02129	0.9877

TABLE III

COMPARISON OF EXPERIMENTALLY FOUND, ECL_F, AND PUBLISHED, ECL_P, DATA FOR FAMEs SEPARATED ON SP 2340 STATIONARY PHASE

FAME	*170°C*		*200°C*	
	ECL_F	ECL_P^4	ECL_F	ECL_P^5
iso-14:0	13.52	13.43	13.47	13.52
aiso-15:0	14.70	14.56	14.71	14.74
iso-16:0	15.52	15.43	15.47	15.52
16:1ω7t	16.46	16.61	16.55	16.52
16:1ω7c	16.66	16.89	16.79	16.73
aiso-17:0	16.70	–	16.71	16.72
18:1ω9t	18.37	18.54	18.46	18.37
18:1ω7t	18.42	18.56	18.48	–
18:1ω9c	18.53	18.77	18.67	18.62
18:1ω7c	18.70	18.84	18.73	18.70
18:2ω6t,t	19.01	19.31	19.04	19.05
18:2ω6c,c	19.34	19.71	19.60	19.47
20:1	20.44	20.77	20.62	20.55
20:2ω6c,c	21.19	21.71	21.47	21.41
22:1ω9t	22.28	22.54	22.36	22.30
22:1ω9c	22.39	22.47	22.50	22.50

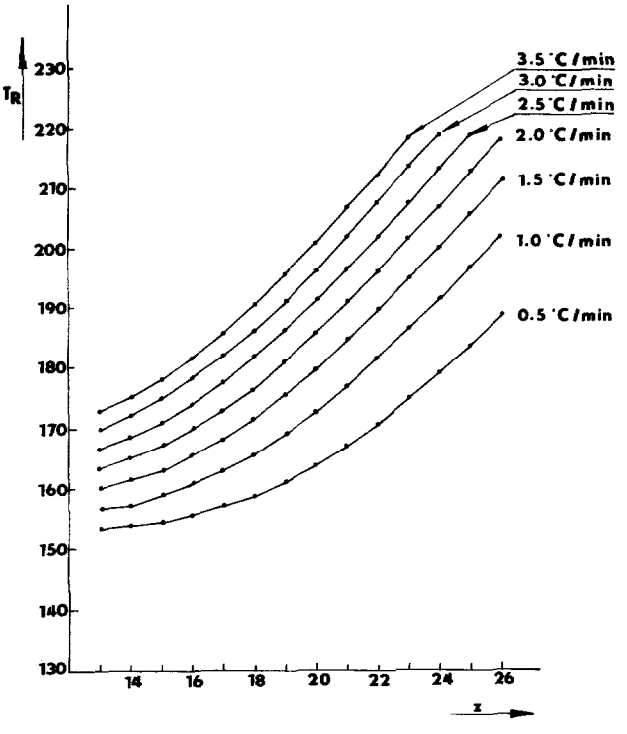

Fig. 2. Dependence of the elution temperatures, T_R, of normal saturated FAMEs on the number of carbon atoms, z, in their alkyl chains.

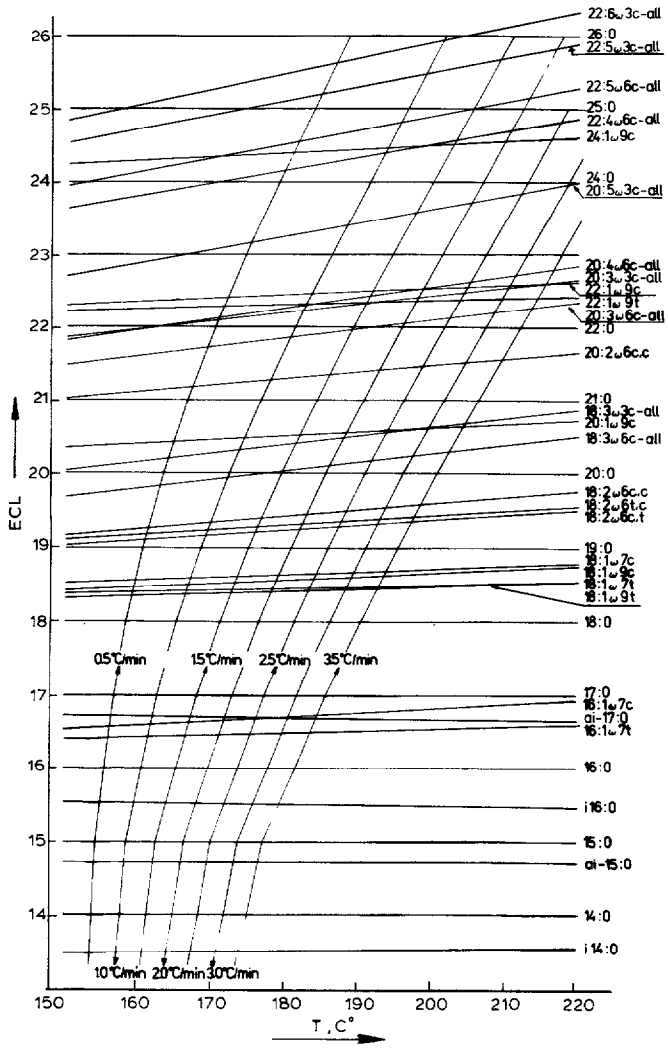

Fig. 3. Graph for prediction of elution temperatures in LTPCGC, plotted using data from Table II and Fig. 2.

of least squares for FAMEs separated on SP 2340 at 150–220°C. From the correlation coefficients, r, it can be concluded that eqn. 3 is not valid for branched FAMEs. Also, that the values of the slopes B increase with increasing number of double bonds. The slopes for monoenic FAMEs are higher for the *cis* than for the *trans* isomers, in agreement with our previously published results on other types of liquid phases[8].

The use of published ECL data for the tentative identification of FAMEs is very difficult since the reproducibility is very poor, as can be seen from Table III. In order to improve the reproducibility, standardization of experiment conditions, e.g., stationary film thickness and polarity of the capillary walls, will be necessary.

It was reported that in LTPGC there exists a linear relationship between the

TABLE IV

COMPARISON BETWEEN PREDICTED, $T_{R,p}$, AND EXPERIMENTAL, $T_{R,f}$, ELUTION TEMPERATURES OF FAMEs SEPARATED ON SP 2340 USING DIFFERENT TEMPERATURE GRADIENTS

FAME	*0.5°C/min*		*1.0°C/min*		*1.5°C/min*		*2.0°C/min*		*2.5°C/min*		*3.0°C/min*		*3.5°C/min*	
	$T_{R,p}$	$T_{R,f}$	$T_{R,p}$	$T_{R,f}$	$T_{R,p}$	$T_{R,f}$	$T_{R,p}$	$T_{R,f}$	$T_{R,p}$	$T_{R,f}$	$T_{R,p}$	$T_{R,f}$	$T_{R,p}$	$T_{R,f}$
aiso-15:0	154.5	154.6	158.9	158.8	162.9	162.8	166.6	166.5	170.3	170.2	174.0	173.9	177.4	177.3
iso-16:0	155.3	155.3	160.2	160.1	164.6	164.5	168.6	168.6	172.6	172.5	176.5	176.5	180.0	180.1
16:1ω7t	156.4	156.3	161.9	161.8	166.9	166.7	171.4	171.2	175.7	175.5	180.0	179.7	183.9	183.6
16:1ω7c	156.6	156.6	162.3	162.2	167.4	167.2	172.0	171.8	176.5	176.1	180.9	180.5	184.8	184.4
aiso-17:0	156.7	156.7	162.5	162.4	167.4	167.5	171.9	172.1	176.3	176.4	180.6	180.8	184.5	184.7
18:1ω9t	159.6	159.5	167.0	166.8	173.2	173.0	178.6	178.4	183.6	183.2	188.5	188.1	192.8	192.4
18:1ω7t	159.8	159.7	167.1	167.0	173.4	173.2	178.8	178.6	183.7	183.5	188.6	188.3	193.0	192.7
18:1ω9c	159.9	159.8	167.4	167.2	173.8	173.5	179.3	178.9	184.3	183.9	189.4	188.8	193.7	193.2
18:1ω7c1	160.2	160.0	167.7	167.5	174.1	173.8	179.6	179.3	184.6	184.3	189.7	189.2	194.0	193.6
18:2ω6t,t	161.0	160.9	169.0	168.9	175.6	175.4	181.2	181.2	186.3	186.2	191.5	191.3	195.8	195.7
18:2ω6c,t	161.4	161.3	169.6	169.4	176.4	176.1	182.4	181.8	187.6	187.0	193.0	192.2	197.4	196.6
18:2ω6t,c	161.4	161.5	169.6	169.6	176.4	176.4	182.4	182.2	187.6	187.4	193.0	192.6	197.4	197.0
18:2ω6c,c	161.8	161.6	170.3	169.9	177.4	176.7	183.2	182.5	188.6	187.8	194.1	193.0	198.7	197.5
18:3ω6c-all	163.4	163.1	172.6	172.0	180.2	179.3	186.4	185.4	192.2	190.8	197.8	196.1	202.7	200.9
18:3ω3c-all	164.4	164.1	174.5	173.5	181.5	180.9	188.4	187.2	194.2	192.7	199.9	198.2	204.7	202.9
20:1ω9c	165.2	164.9	174.8	174.4	182.4	181.9	188.8	188.1	194.3	193.6	199.8	199.0	204.5	203.6
20:2ω6c,c	167.6	167.4	178.3	177.7	186.4	185.6	193.2	192.1	198.6	197.8	204.8	203.5	209.7	208.2
20:3ω6c-all	169.7	169.2	181.0	180.0	189.6	188.6	196.5	195.0	202.1	200.9	208.6	206.8	213.8	211.6
20:3ω3c-all	171.2	170.6	182.7	181.8	191.4	190.2	198.4	197.0	204.5	202.9	210.7	208.8	215.7	213.7
20:4ω6c-all	171.3	170.6	183.0	181.9	191.9	190.4	199.0	197.2	205.4	203.3	211.6	209.3	217.1	214.1
22:1ω9t	171.8	171.7	183.2	182.9	191.4	191.1	198.2	197.8	203.4	203.6	209.8	209.3	214.6	214.1
22:1ω9c	172.4	172..1	183.9	183.3	192.3	191.7	199.1	198.4	204.5	203.6	210.8	209.3	215.7	214.1
20:5ω3c-all	175.6	174.4	188.6	186.6	198.0	195.5	205.4	202.7	212.0	208.9	218.6	215.5		
22:4ω6c-all	179.9	179.0	193.6	191.9	203.3	201.0	210.7	208.3	217.6	214.6				
24:1ω9c	181.0	180.6	194.0	193.3	203.0	202.2	210.0	209.1	216.3	215.2				
22:5ω6c-all	181.8	180.6	196.0	193.7	205.8	203.0	213.4	210.3	220.0	216.7				
22:5ω3c-all	185.0	183.2	199.4	196.7	209.4	206.2	217.1	213.6						
22:6ω3c-all	187.1	185.0	201.6	198.7	212.0	208.4	220.0	215.8						

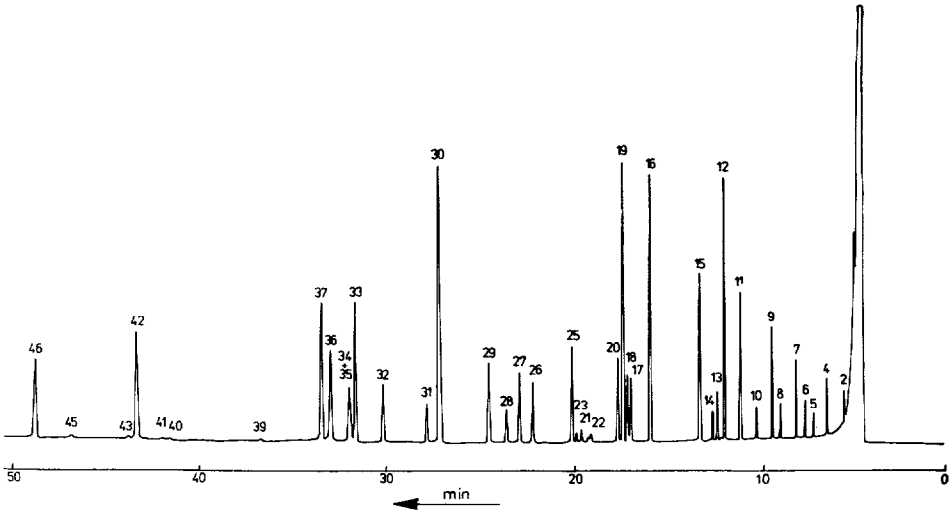

Fig. 4. Separation of FAMEs on a SP 2340 glass capillary column using LTPGC from 150 to 220°C with a gradient of 1°C/min. For other conditions, see Experimental. For peak identification, see Fig. 1.

retention times and the carbon numbers of homologous series[11]. Since the retention time, t_R, in LTPGC corresponds to the elution temperature, T_R, we could expect a linear relationship between the latter and the carbon number, z, for the alkyl chain of normal saturated FAMEs. From Fig. 2 it is seen that this dependence is not linear when FAMEs are separated on SP 2340 as stationary phase. The slopes of the lines change with the temperature gradient as well as with the carbon number, z. This is why we do not use "ECL values calculated in the LTPGC conditions" for the purposes of tentative identification of FAMEs separated by LTPGC.

Knowing the isothermal ECL values (Table II) and the retention temperatures, T_R, of normal saturated FAMEs, we were able to predict the retention temperatures of branched and unsaturated FAMEs using eqn. 4. Table IV compares predicted, $T_{R,p}$, and experimental $T_{R,f}$, elution temperatures of FAMEs separated on SP 2340 by LTPCGC using different temperature gradients. There is good agreement between these values for branched and monoenic FAMEs, the differences being less than 1.2°C. The largest difference, $T_R = T_{R,p} - T_{R,f}$, was found for 22:6ω3c-all, namely 4.2°C.

We have found that for the prediction of retention temperature a graphic procedure can be used as shown in Fig. 3, where the dependences of isothermal ECL values on temperature (data from Table II) as well as of retention temperatures of normal saturated FAMEs on the carbon number (data from Fig. 2) are given. It can be concluded that the elution order of FAMEs can be dramatically changed by changing the temperature gradient in LTPCGC. For example, the separation of FAMEs using LTPGC with a gradient of 1°C/min is shown on Fig. 4. A comparison of Figs. 1 and 3 demonstrated the improved separation of pairs 13,14 and 17,18, but the pair 34,35 is still not resolved. From Fig. 3 the optimum gradient for LTPCGC can be found by computation[12].

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