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● GAS CHROMATOGRAPHY
No.G252

Analysis of 37 FAMEs using 6 Types of Capillary Columns

Fatty acids are the main constituents of fats that are contained in foods, and they are categorized according to the number of unsaturated bonds in their chemical structure, as described below.

Saturated fatty acid, SFA
  Fatty acid with no unsaturated bonds in the carbon chain
Monounsaturated fatty acid, MUFA
  Fatty acid with one unsaturated bond in the carbon chain
Polyunsaturated fatty acid, PUFA
  Fatty acid with multiple unsaturated bonds in the carbon chain

In addition, polyunsaturated fatty acids (PUFAs) are further classified into the following two types, depending upon the position of the double bond closest to the ester group:
n-3 (also notated as ω-3) system
n-6 (ω-6) system

These classifications are based on the position of the double bond between the carbons, in which the first double carbon bond is the third carbon-carbon bond from the fatty acid methyl terminal (opposite end from the carboxyl terminal) in the n-3 system, and the sixth from the methyl terminal in the n-6 system.
As the roles of fatty acids in the body vary depending on their structure, it is necessary to conduct detailed compositional analysis of fatty acids contained in foods.

Higher fatty acids, those having a large number of carbon atoms, are generally analyzed by GC after undergoing methyl esterification (fatty acid methyl esters, FAME).
This Application News introduces an analysis of 37 kinds of fatty acid methyl esters (FAME) using 6 types of capillary columns designated for FAME analysis.
The naming convention for FAMEs in this article is "C (number of carbons) : (number of double bonds) n (position of nearest double bond to methyl group)".

■ Capillary Columns and Analytical Conditions

The columns used in the investigation are as follows (from lower to higher polarity).

FAMEWAX (Polyethylene glycol: bonded-phase)
BPX70 (70 % biscyanopropyl polysilphenylene-siloxane: bonded-phase)
HR-SS-10 (Nitrile silicone: non-bonded-phase)
Rtx-2330 (90 % biscyanopropyl / 10 % phenylcyanopropyl-polysiloxane: non-bonded-phase)
BPX90 (90 % biscyanopropyl polysilphenylene-siloxane: bonded-phase)
RT-2560 (90 % biscyanopropyl-polysiloxane : non-bonded-phase)

Figs.2 through 7 show chromatograms obtained in the respective temperature program analyses. As the column temperature programming conditions vary depending on the column, the respective conditions are shown below in figures. Several analytical conditions (other than those associated with the column temperature program) were held constant, and are as follows.

Table 1 GC Analytical Conditions

Model	: GC-2010
Inj.Temp.	: 250 ˚C
Carrier Gas	: He 25 cm/sec (Constant Linear Velocity Mode)
Split Ratio	: 1:25

■ Equivalent Chain Length (ECL) of C20 FAMEs at 180 ˚C (Isothermal Analysis)

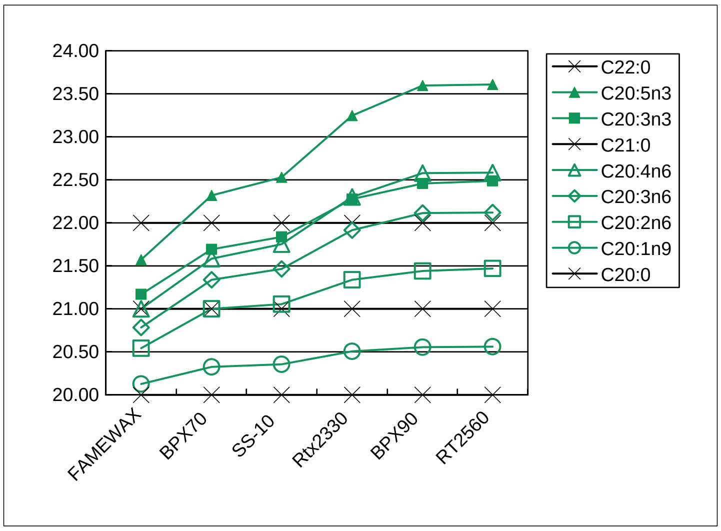


Fig.1 ECLs of C20 FAMEs at 180 ˚C (Isothermal Analysis)

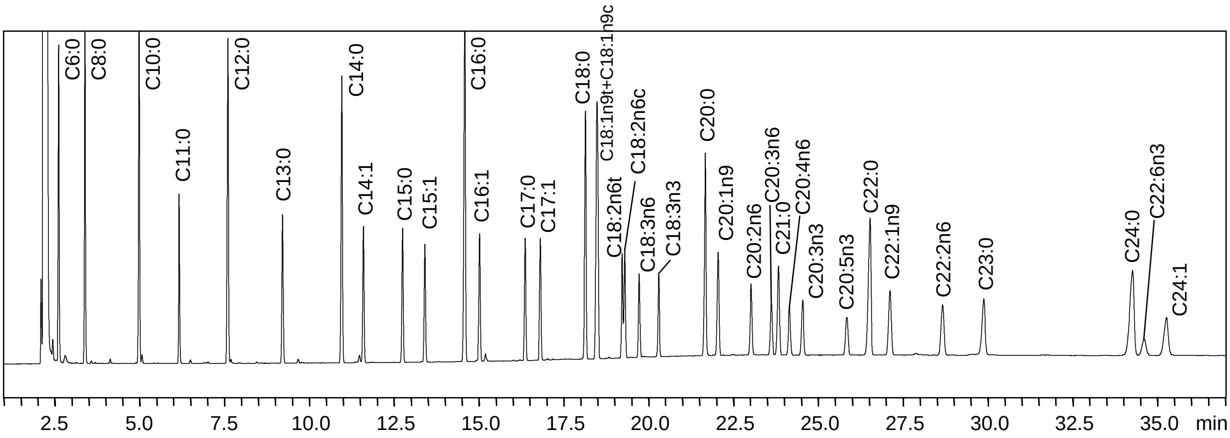
The equivalent chain length (ECL) relative to saturated, straight-chain fatty acid methyl esters is an indication of the relative retention time based on the corresponding saturated fatty acid.
The retention time of a saturated fatty acid having N carbons is assumed to be N (for example, C20 : 0 elution time is 20.0), and indicates the relative elution position of each FAME.

Fig.1 shows the ECLs of fatty acid methyl esters with 20 carbons at 180 ˚C isothermal analysis for each column.

The higher the polarity of the liquid phase, the longer the elution time becomes for highly unsaturated fatty acids.

Table 2 shows ECLs of C14 to C24 FAMEs at 180 ˚C isothermal analysis.

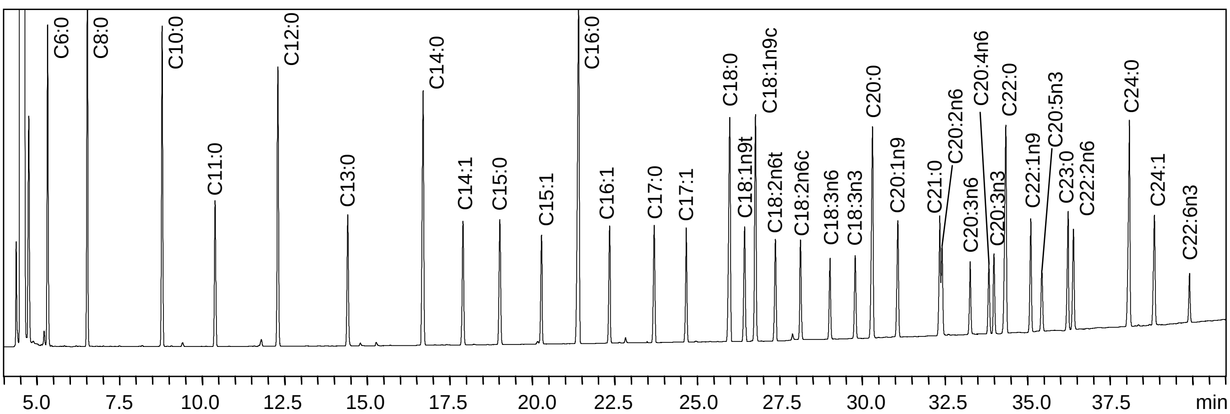
■ FAME WAX (Polyethylene Glycol : Bonded-Phase)



Column : FAMEWAX 30m × 0.25 mm I.D. df : 0.25 µm Column Temp : 130 ˚C (0 min)--230 ˚C at 5 ˚C/min

Fig.2

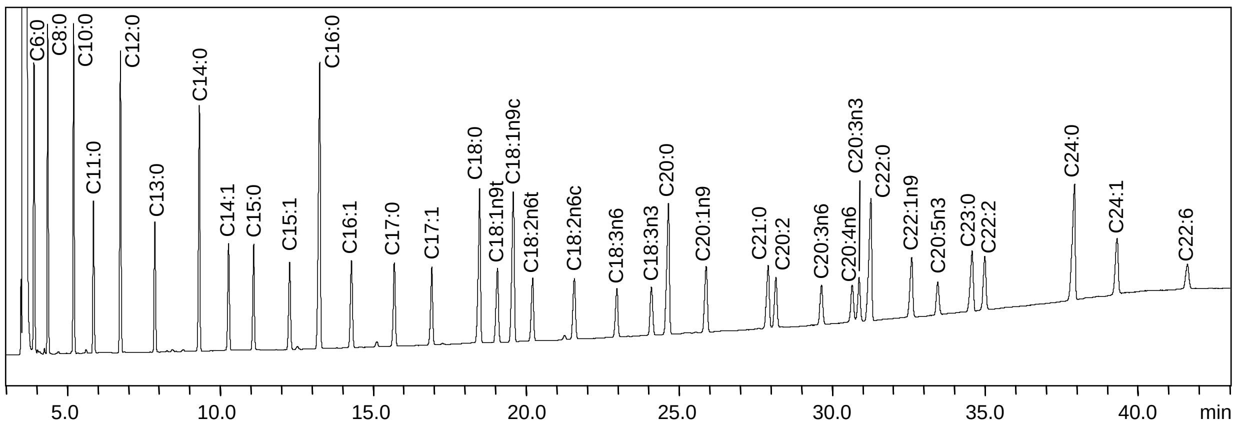
■ BPX70 (70 % Cyanopropyl Polysilphenylene-Siloxane : Bonded-Phase)



Column : BPX70 60m × 0.22 mm I.D. df : 0.25 µm Column Temp : 120 ˚C (0 min)--250 ˚C at 3 ˚C/min

Fig.3

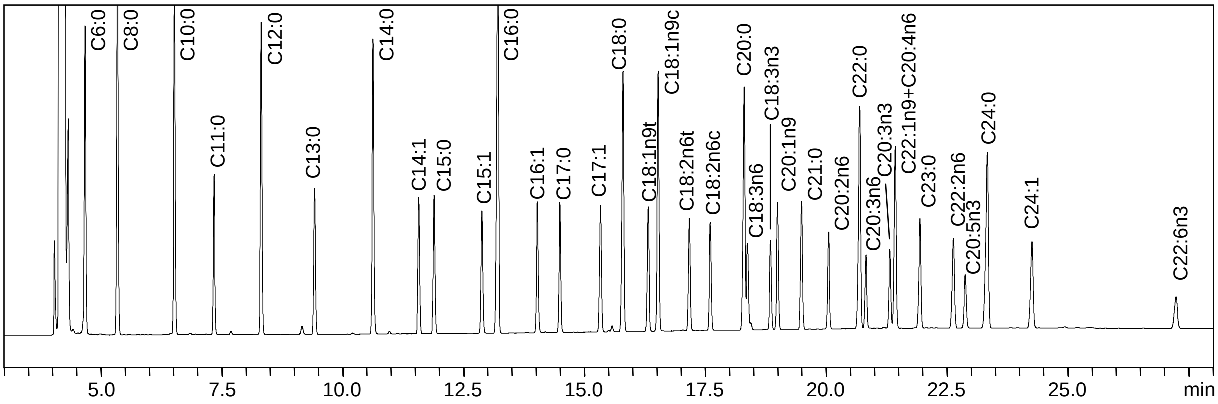
■ HR-SS-10 (Nitril Silicone : Non-Bonded-Phase)



Column : HR-SS-10 50 m × 0.25 mm I.D. Column Temp : 160 ˚C (0 min)--220 ˚C at 1.5 ˚C/min

Fig.4

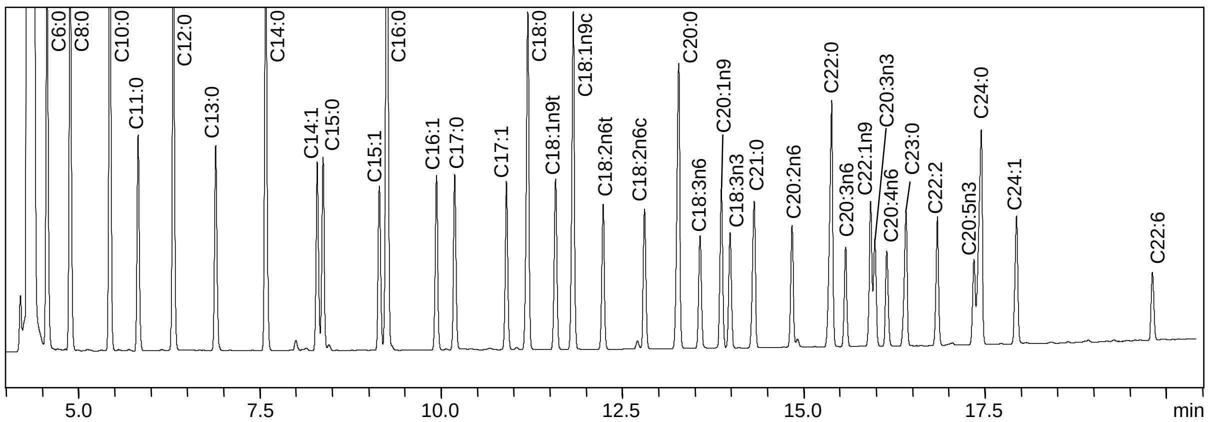
■ Rtx-2330 (90 % Biscyanopropyl 10 % Cyanopropylphenyl Polysiloxane : Non-Bonded-Phase)



Column : Rtx-2330 60 m × 0.25 mm I.D. df:0.20 µm Column Temp : 130 ˚C (0 min)--230 ˚C at 5 ˚C/min

Fig.5

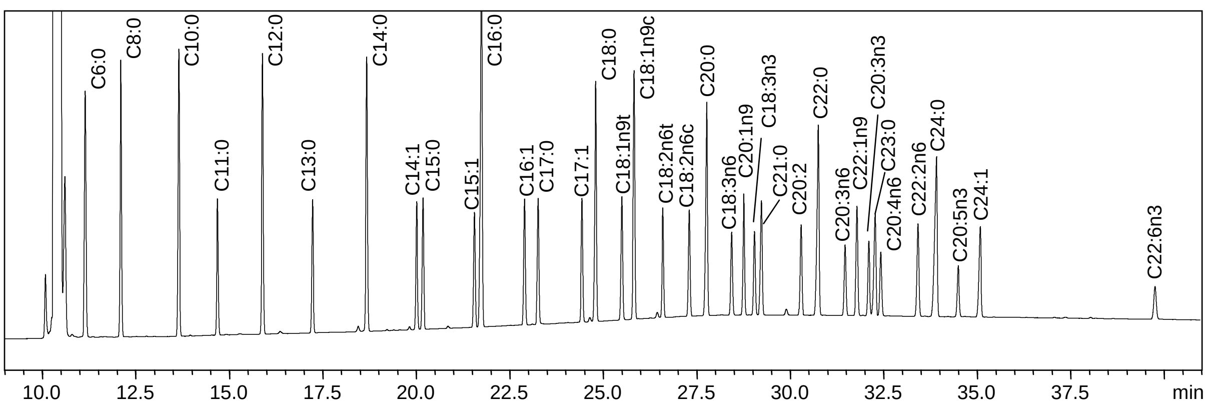
■ BPX90 (90 % Cyanopropyl Polysilphenylene-Siloxane : Bonded-Phase)



Column : BPX90 60 m × 0.25 mm I.D. df : 0.25 µm Column Temp : 140 ˚C (0 min)--260 ˚C at 5 ˚C/min

Fig.6

■ RT-2560 (100 % Biscyanopropyl Polysiloxane : Non-Bonded-Phase)



Column : RT-2560 100 m × 0.25 mm I.D. df : 0.20 µm Column Temp : 140 ˚C (2 min)--240 ˚C at 4 ˚C/min

Fig.7

■ ECLs of C14 to C24 FAMEs at 180 °C (Isothermal Analysis)

Table 2 ECLs of C14 to C24 FAMEs at 180 ˚C (Isothermal Analysis)

	FAMEWAX	BPX70	SS-10	Rtx2330	BPX90	RT2560
C14:1	14.34	14.57	14.57	14.81	15.00	14.94
C15:0	15.00	15.00	15.00	15.00	15.00	15.00
C15:1	15.33	15.57	15.58	15.80	16.00	15.92
C16:0	16.00	16.00	16.00	16.00	16.00	16.00
C16:1	16.22	16.44	16.45	16.66	16.81	16.76
C17:0	17.00	17.00	17.00	17.00	17.00	17.00
C17:1	17.21	17.43	17.45	17.64	17.78	17.74
C18:0	18.00	18.00	18.00	18.00	18.00	18.00
C18:1n9t	18.12	18.17	18.17	18.33	18.34	18.37
C18:1n9c	18.14	18.30	18.31	18.47	18.56	18.54
C18:2n6t	18.46	18.52	18.49	18.95	18.93	19.04
C18:2n6c	18.51	18.86	18.93	19.30	19.55	19.50
C18:3n6	18.71	19.29	19.40	20.00	20.31	20.25
C18:3n3	19.04	19.69	19.81	20.34	20.69	20.62
C20:0	20.00	20.00	20.00	20.00	20.00	20.00
C20:1n9	20.12	20.33	20.36	20.50	20.55	20.56
C20:2n6	20.54	21.00	21.06	21.34	21.44	21.47
C20:3n6	20.78	21.34	21.46	21.92	22.11	22.12
C20:3n3	21.17	21.69	21.84	22.28	22.46	22.49
C20:4n6	21.00	21.59	21.75	22.30	22.58	22.58
C20:5n3 (EPA)	21.58	22.32	22.53	23.25	23.59	23.61
C21:0	21.00	21.00	21.00	21.00	21.00	21.00
C22:0	22.00	22.00	22.00	22.00	22.00	22.00
C22:1n9	22.12	22.31	22.35	22.47	22.46	22.50
C22:2n6	22.54	22.96	23.06	23.28	23.27	23.37
C22:6n3 (DHA)	23.85	24.72	25.12	26.34	26.64	26.99
C23:0	23.00	23.00	23.00	23.00	23.00	23.00
C24:0	24.00	24.00	24.00	24.00	24.00	24.00
C24:1n9	24.16	24.39	24.45	24.56	24.46	24.59

With all the columns:
- When the number of carbon atoms in a molecule is the same, the elution time becomes longer as the number of double bonds becomes greater.
- If both the carbon number and the number of double bonds are the same, elution is slower with n-3 than with n-6 FAMEs.
(Comparison of C18 : 3n3 and C18 : 3n6, C20 : 3n3 and C20 : 3n6)

With respect to the differences between the columns, just as with the C20 FAMEs in Fig.1, the C14 to C24 FAME compounds listed above showed the same tendency; that is, the higher the polarity of the liquid phase, the longer the elution time becomes for highly unsaturated fatty acids.

Fig.1 and Table 2 show the ECLs for isothermal analysis at 180 ˚C.
The ECLs also vary depending on the column temperature program conditions.
Generally, if the column temperature is increased, the ECLs of unsaturated fatty acid methyl esters become greater (elution becomes relatively longer).
As the degree of fatty acid unsaturation in a given molecule increases and the column liquid phase polarity increases, that trend becomes even greater.

NOTES:
*This Application News has been produced and edited using information that was available when the data was acquired for each article. This Application News is subject to revision without prior notice.

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