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Quick Separation of the Main Components of Fatty Acid Methyl Esters by Capillary Gas Chromatography: What Is the Shortest Time?

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1 Introduction

Fatty acid profiles (showing the fatty acid content) of vegetable, fish and animal oils are determined by gas chromatography (GC), after converting the oils to fatty acid methyl esters (FAMEs).[1] Getting these profiles is an important job in lipid chemistry and also in the food industry. Before running the GC instrument, the analyst decides on the following: the kind of column, the column temperature (C.Temp.), the kind of carrier gas and the linear gas velocity (L.G.Vel.). The amount of time it takes for the GC run and the precision of the fatty acid profiles depend on these four variables. To illustrate, at a research center, an analyst may use a longer length column with high-resolution[2-6] and a standard L.G.Vel., resulting in a long GC run time, to get a detailed fatty acid profile showing all the components of the FAMEs; but at a food manufacturing plant, an analyst may increase the L.G.Vel. and the C.Temp., resulting in a short GC run time, to get a fatty acid profile showing only the main components of the FAMEs. Some analysts in manufacturing want the shortest possible GC run time that gives a fatty acid profile showing only the information they need.[7] We developed a model system that does this.

In this communication, we describe and compare the usual system for capillary GC and our model system for high-speed capillary GC, with both systems using a standard flexible fused-silica (FFS) capillary column[2,3,8] (the DB-23 column, commercially available). We then describe how we applied our model system and determined the maximum operating conditions to analyze the main components of FAMEs from soybean oil in the shortest amount of time.

2 Materials and Methods

2.1 Chemicals and preparation of FAMEs

Standard fatty acids, 16:0, 18:0, 18:1(9), 18:2(9,12), 18:3(9,12,15) and 20:0 were obtained from Wako Pure Chemical Industries (Osaka, Japan), and 20:1(11) and 24:1(15) were from Nu-Chek Prep (Elysian, MN). Our laboratory standard FAME mixture (prepared from mango pulp lipids)[9,10] was also used. Other chemicals were of the highest reagent grade available, and all the solvents were distilled before use.

Standard fatty acids were methylated with 14% BF_3/methanol,[9] and soybean oil was converted to FAMEs by methanolysis (0.5 mol/L KOH/methanol) and successive methylation (14% BF_3/methanol).[9] These FAMEs were purified by thin-layer chromatography[9] before GC injection.

2.2 Capillary GC conditions

A standard FFS capillary column, a narrow bore DB-23 column (30 m × 0.25 mm i.d., 0.25 μm film thickness), was borrowed from Yokogawa Analytical Systems (Tokyo, Japan). FAMEs were separated on the DB-23 column linked to a Shimadzu GC-17A gas chromatograph with a split/splitless injector, a flame ionization detector and a Shimadzu work station on-line system (Class-GC10). Temperature of the injector and detector ports was maintained at 270℃. Helium (purity, 99.999%) or hydrogen (purity, over 99.9%) was used as the carrier gas without purifying. Other operating conditions are given in the corresponding Figures and Tables.

3 Results and Discussion

3.1 Usual system for capillary GC

First we analyzed our laboratory standard FAME mixture,[9,10] containing several types of double-bond positional isomers, under the usual operating conditions to check the quality of our DB-23 capillary column (a procedure that must be done before using a new column). Fig. 1 shows the base-line separations of the double-bond positional isomers: 16:1(9) and 16:1(11), and 18:1(9) and 18:1(11). These isomers separated with high resolution, and all the components of the FAMEs eluted clearly. Our column worked just as typical high-resolution capillary columns do.[2-6]

The usual operating conditions, when using helium (or hydrogen) as the carrier gas, were as follows: the C.Temp., 175℃; the L.G.Vel., 30 cm/sec. Under these usual operating conditions the time of the GC run was within 15 min, as shown in Fig. 1.

3.2 Our model system for capillary GC

We prepared an original test mixture of FAMEs composed of nearly equal amounts of authentic methyl 18:3(9,12,15), methyl 20:0, methyl 20:1(11) and methyl 24:1(15). We selected these four components of FAMEs for

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Fatty acid abbreviations: myristic acid, 14:0; myristoleic acid, 14:1(9); palmitic acid, 16:0; palmitoleic acid, 16:1(9); *cis*-11-hexadecenoic acid, 16:1(11); stearic acid, 18:0; oleic acid, 18:1(9); *cis*-vaccenic acid, 18:1(11); linoleic acid, 18:2(9,12); α-linolenic acid, 18:3(9,12,15); arachidic acid, 20:0; gondoic acid, 20:1(11); nervonic acid, 24:1(15).

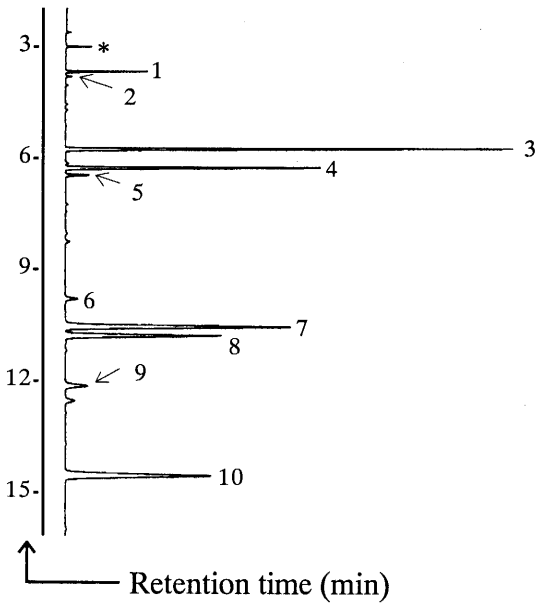

Fig. 1 Capillary gas chromatogram, using the DB-23 column, of fatty acid methyl esters prepared from mango pulp lipids.
1=14:0, 2=14:1(9), 3=16:0, 4=16:1(9), 5=16:1(11), 6=18:0, 7=18:1(9), 8=18:1(11), 9=18:2(9,12), 10=18:3(9,12,15). Column temperature, 175℃; carrier gas, helium at a linear gas velocity of 30 cm/sec (split ratio of 1/25). BHT (butylated hydroxy toluene) was added to the sample as an antioxidant, and it (*) elutes at 3 min.

the following reasons. On the DB-23 column, methyl 18:3(9,12,15) elutes before methyl 20:0 under the usual operating conditions; however, when the GC is running at a certain higher speed than the usual operating speed these FAMEs co-elute, making it impossible to determine their amounts, and therefore, we can judge that the running speed of the GC is too fast. This is also true when methyl 20:0 and methyl 20:1(11) co-elute. Because 24:1(15) is, in general, the acid with the longest carbon chain in natural lipids, the retention time of methyl 24:1(15) is the maximum amount of time it takes to determine all the components of FAMEs. We used the DB-23 column to find out what the shortest time it takes to separate these four main components of the FAMEs.

We tested our model system with helium as the carrier gas and then, with hydrogen. In Japan, hydrogen is seldom used as a carrier gas; but in Europe and the US, both helium and hydrogen are commonly used. The efficiency of our model system depends on the carrier gas and so we compared how well these two gases worked.

We first used helium (Table 1, upper part). The L.G.Vel. was constant at 30 cm/sec as we increased the C.Temp. (from 240 to 250℃). We got shorter retention times of the four components of the FAMEs as the C.Temp. increased. Methyl 18:3(9,12,15) and methyl 20:0 co-eluted at the C.Temp. of 250℃. We then analyzed our original test mixture at a constant L.G.Vel. of 40 cm/sec and then at a constant L.G.Vel. of 50 cm/sec as we increased the C.Temp. (from 240 to 250℃). The co-elutions also occurred at 250℃. At last, we determined that a L.G.Vel. of 77 cm/sec and a C.Temp. of 240℃ are the maximum operating conditions when using helium for analyzing a FAME sample containing methyl 18:3(9,12,15) and methyl 20:0. The GC run time was within 2 min. Because the pressure limit for any carrier gas in the Shimadzu GC-17A is at 400 kPa, getting the L.G.Vel. over 77 cm/sec while using helium is impossible.

Table 1 Retention times of fatty acid methyl esters in our original test mixture on the DB-23 capillary column under the various conditions

L.G.Vel. (cm/sec)	C.Temp. (℃)	Fatty acid and retention time (min) 18:3 (9,12,15)	20:0	20:1 (11)	24:1 (15)
Carrier gas: helium					
30	240	2.83	2.88	3.00	4.87
	245	2.66	2.69	2.79	4.31
	250	2.50	Co.E.	2.62	3.84
40	240	2.16	2.20	2.30	3.72
	245	2.02	2.05	2.13	3.28
	250	1.90	Co.E.	1.99	2.93
50	240	1.74	1.77	1.85	2.99
	245	1.63	1.66	1.72	2.65
	250	1.54	Co.E.	1.61	2.36
77[a]	240	1.11	1.13	1.17	1.88
Carrier gas: hydrogen					
30	240	2.88	2.95	3.06	4.95
	245	2.75	2.79	2.89	4.43
	250	2.59	2.62	2.71	3.96
40	240	2.17	2.21	2.30	3.71
	245	2.06	2.09	2.16	3.32
	250	1.95	1.97	2.04	2.98
50	240	1.76	1.79	1.87	3.00
	245	1.65	1.68	1.74	2.67
	250	1.57	Co.E.	1.65	2.40
60	240	1.47	1.50	1.56	2.51
80	240	1.11	1.13	1.18	1.90
120	240	0.76	0.78	0.81	1.30
177[a]	240	0.51	Co.E.	0.54	0.87

The split ratio is 1/25 for both the carrier gases. Each value is an average of three determinations. L.G.Vel., linear gas velocity. C.Temp., column temperature. Co.E., co-elution with 18:3(9,12,15).
[a] limit of linear gas velocity (pressure: 400 kPa) in the GC instrument.

With hydrogen as the carrier gas (Table 1, lower part), we increased the L.G.Vel. as we increased the C.Temp., just as we did with helium. We got shorter retention times of the four components of FAMEs as the L.G.Vel. and C.Temp. increased. Methyl 18:3(9,12,15) and methyl 20:0 co-eluted at the C.Temp. of 250℃ and with the L.G.Vel. of 50 cm/sec. We then lowered the C.Temp. (240℃) and increased the L.G.Vel. (from 60 to 177 cm/sec.). We determined that a L.G.Vel. of 177 cm/sec and a C.Temp. of 240℃ are the maximum operating conditions when using hydrogen to analyze a FAME sample containing methyl 18:3(9,12,15) and methyl 20:0. The GC run time was 1.30 min. Any GC system with hydrogen separates compounds better than a system with helium because the molecular weight of hydrogen is lower than that of helium.[11] We could use a higher L.G.Vel. than the limit of 77 cm/sec for helium.

Because we found that using hydrogen as the carrier gas gave us the most efficient system, we used it to analyze the main components of soybean oil FAMEs.

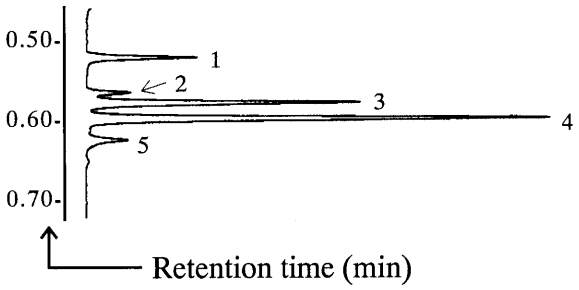

Fig. 2 Capillary gas chromatogram, using the DB-23 column, of fatty acid methyl esters prepared from soybean oil. 1=16:0, 2=18:0, 3=18:1(9) + a small amount of 18:1(11), 4=18:2(9,12), 5=18:3(9,12,15) + a small amount of 20:0. Column temperature, 250℃; carrier gas, hydrogen at a linear gas velocity of 125 cm/sec (split ratio of 1/40).

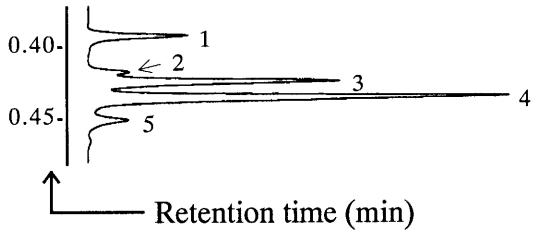

Fig. 3 Capillary gas chromatogram, using the DB-23 column, of fatty acid methyl esters prepared from soybean oil. 1=16:0, 2=18:0, 3=18:1(9) + a small amount of 18:1(11), 4=18:2(9,12), 5=18:3(9,12,15) + a small amount of 20:0. Column temperature, 250℃; carrier gas, hydrogen at a linear gas velocity of 177 cm/sec (split ratio of 1/20).

3.4 Analysis of soybean oil fatty acid profile with our model system

Natural oils, unlike our original test mixture, contain small amounts of minor components of fatty acids. In lipid chemistry, we want to know these exact amounts. When they co-elute with the main components is important information. A long GC run at a standard temperature is necessary and so our high-speed model system cannot be used for this kind of analysis. In the food industry, however, it is unnecessary to determine the small amounts of minor components when checking the quality of common oils. It is only necessary to check the main components of the fatty acids. The point at which the minor components co-elute with the main components can be ignored. And so, checking these co-eluted amounts of the main components is a standard procedure. Our high speed model system can be successfully used for this kind of analysis. We wanted to see how fast a GC run we could get when analyzing soybean oil FAMEs. We set the GC instrument to the maximum C.Temp. (250℃). We increased the L.G.Vel. to 125 cm/sec and got the run time of 40 sec, with the main components of the soybean oil FAMEs clearly separating, as shown in Fig. 2. The value of this L.G.Vel. is 95 cm/sec greater than the L.G.Vel. used in the usual system for capillary GC.

We then increased the L.G.Vel. to 177 cm/sec (at 400 kPa, the maximum pressure of the GC instrument). Fig. 3 shows the gas chromatogram. This GC run was completed within 30 sec. Methyl 18:0 (peak 2) and methyl 18:1(9) (peak 3) still separate, but not as clearly as they do as shown in Fig. 2.

Table 2 shows data that supports our work. The data of the fatty acid profiles for the main components of the soybean oil FAME sample are similar, whether they were determined using the usual system for capillary GC (Fig. 1) or using our model system with hydrogen (Figs. 2 and 3). With our model system, the main components of 18:1(9) and 18:3(9,12,15) co-elute with the minor components of 18:1(11) and 20:0 (operating conditions B and C in Table 2). Because of this co-elution, the total amounts of 18:1(9) and of 18:3(9,12,15) are higher than those we measured when using the usual system (operating condition A in Table 2) in which the minor components separately elute. These data are cited without any statistical treatment.

3.5 Conclusion and our recommendation

When using our model system (Table 1), the main components of fatty acids of common vegetable oils, in which methyl 18:3(9,12,15) is the last peak to elute in GC analysis, can be analyzed in 1.2 min with helium and in 0.8 min with hydrogen. Rapeseed oil or peanut oil, having fatty acids with longer carbon chains, can be analyzed in 2 min with helium and in 1.5 min with hydrogen [because methyl 24:1(15) elutes at 1.88 min with helium and at 1.30 min with hydrogen]; this is also true for fish and animal oils, containing docosahexaenoic acid, since an equivalent chain-length value[12] of methyl docosahexaenoic acid is less than 24.0 (data not shown).

Our research group recommends using hydrogen as the

Table 2 Comparing fatty acid profiles of soybean oil samples determined by capillary GC with the DB-23 column using hydrogen as the carrier gas under the three sets (A, B, C) of operating conditions

Operating condition	Fatty acid (wt%)							No. of GC run
	16:0	18:0	18:1 (9)	18:1 (11)	18:2 (9,12)	18:3 (9,12,15)	20:0	
A	11.2	4.6	27.4	1.6	49.9	4.6	0.7	n=10
B	10.6	4.2	29.1[a]	-	50.5	5.6[b]	-	n=14
C	10.7	4.3	29.3[a]	-	49.7	6.0[b]	-	n=5

Each value is an average of the repeated GC runs indicated (n).
Operating condition A: column temperature, 175℃; linear gas velocity, 30 cm/sec (see Fig. 1).
Operating condition B: column temperature, 250℃; linear gas velocity, 125 cm/sec (see Fig. 2).
Operating condition C: column temperature, 250℃; linear gas velocity, 177 cm/sec (see Fig. 3).
[a] including the co-eluted 18:1(11).
[b] including the co-eluted 20:0.

carrier gas, instead of helium, for the following reasons. The cost of hydrogen is cheaper. The efficiency of the column is less dependent on the L.G.Vel. when using hydrogen.[11] We can, therefore, use a higher L.G.Vel., resulting in a shorter GC run time. During the one year we used this system, we analyzed a mixture composed of known amounts of saturated FAME and unsaturated FAME just after turning on the GC instrument and just before turning it off. We found that hydrogenation of the unsaturated FAME in the GC instrument did not occur, concurring with a previous review.[11] In addition, using hydrogen is safe with sufficient ventilation in the laboratory and with a GC instrument equipped with an automatic shut-down system in case hydrogen leaks.

For our model GC system the DB-23 column or similar columns must be used. The DB-23 column's polarity, coating (film thickness), thermostability, length, and diameter are suitable for this work. According to the supplier, the maximum C.Temp. is 250℃ (for isothermal use). Unlike a short capillary column,[13,14] it is versatile. We can use it to get a detailed fatty acid profile, and we can also use it to get a fatty acid profile of only the main components of FAMEs in a short time (from 30 or 40 sec to 2 min, depending on the FAMEs).

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