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速 報

Applying Weak Polar Capillary Columns to Analyze Fatty Acid Methyl Esters with Gas Chromatography

Kouhei YAMAMOTO, Akemi KINOSHITA, and Akira SHIBAHARA†

Department of Clinical Nutrition, Osaka Prefecture University, 7-30 Habikino 3-chome, Habikino 583-8555, Japan

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1 Introduction

The introduction of flexible fused-silica (FFS) capillary tubing in 1979 and the early 1980s created a revolution in capillary gas chromatography (GC).[1-3] In the field of lipid chemistry, fatty acid composition of lipids is determined by GC using several types of commercially available FFS capillary columns coated with suitable stationary phases for this purpose.[2-7] The lipids are usually derivatized to fatty acid methyl esters (FAMEs), and the FAMEs are analyzed on medium polar[*1] FFS capillary columns (such as Carbowax 20M, Supelcowax-10, etc.) or polar FFS capillary columns (such as CP-Sil-88, SP-2340, etc.). We have routinely used a medium polar FFS capillary column, ULBON HR-SS-10, for analyzing FAMEs prepared from natural lipids, and those gas chromatograms appear in our previous papers.[8-13]

During our work on sterol analysis of lipids by capillary GC, we preferred to use an SPB-50 column (weak polar). According to the supplier, the SPB-50 column is equivalent to an OV-17 (50% diphenylsiloxane/50% dimethylsiloxane liquid phase) packed-column.[*2] At that time, just by chance, we injected FAMEs onto the SPB-50 column. The unsaturated FAMEs separately eluted just after the corresponding saturated FAMEs with the same carbon chains eluted. The gas chromatogram resembled that which was obtained on a medium polar capillary column. This phenomenon was strikingly contrary to our knowledge: a column equivalent to OV-17 cannot be used for analyzing FAMEs (details given in Section 3).

In this paper we present our examination in which we used weak polar FFS capillary columns equivalent to OV-17 to analyze FAMEs. Though many kinds of stabilized (chemically bonded, cross-linked, or polymerized) FFS capillary columns are now commercially available, non-polar or weak polar columns exhibit superior lifetime (due to the thermostability: above 300℃) over medium and polar columns. This characteristic is most suitable for a column connected to a system of gas chromatography/mass spectrometry (GC/MS), because there is less noise caused by the column bleeding. To our knowledge, the present study is the first to show that a weak polar column can be used for analyzing several types of FAME mixtures by capillary GC.

† Corresponding author. E-mail: shibahar@rehab.osakafu-u.ac.jp

2 Materials and Methods

2.1 FFS capillary columns

A narrow bore SPB-50 column (30 m × 0.25 mm i.d., 0.25 μm film thickness) and a wide bore SPB-50 column (30 m × 0.53 mm i.d., 0.50 μm film thickness) were purchased from Supelco (Bellefonte, PA). A DB-17MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness) and a BPX50 column (30 m × 0.25 mm i.d., 0.25 μm film thickness) were borrowed from Yokogawa Analytical Systems (Tokyo, Japan) and SGE International Pty (Ringwood, Australia), respectively.

2.2 Chemicals

Standard FAME mixtures (2A, 3A and GLC-421-A) were purchased from Nu-Chek Prep (Elysian, MN). Standard fatty acids were obtained from Wako Pure Chemical Industries (Osaka, Japan): palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), and arachidic (20:0) acids. Soybean oil and fish oil capsules (enriched with EPA and DHA) were purchased from a local market. Other chemicals were of the highest reagent grade available, and all the solvents were distilled before use.

2.3 Preparation of FAMEs

Standard fatty acids were methylated with 14% BF_3/methanol.[8] Soybean oil and fish oil capsules were converted to FAMEs by methanolysis (0.5 mol/L KOH/methanol) and successive methylation (14% BF_3/methanol).[8]

2.4 GC conditions

For capillary GC, a Shimadzu GC-17A gas chromatograph with a split/splitless injector and a flame ionization detector was used in combination with a Shimadzu work station on-line system (Class-GC10). The carrier gas was helium at a split ratio of 1/30 (linear gas velocity: 30.0 cm/sec). Other conditions are given in the corresponding figure legends.

Packed-column GC was carried out with a Shimadzu GC-14B equipped with a flame ionization detector and a data-processor (Shimadzu Chromatopac C-R7A). Operating

[*1] There are technical terms expressing column polarity.[5,14-16] In this study, column polarity is classified as non-polar, weak polar, medium polar and polar (see Table 1).

[*2] This packed-column was routinely used to analyze sterols by many lipid chemists until the introduction of FFS capillary columns.[2,14,17]

conditions were as follows: column, 3 m × 3 mm i.d. glass column packed with 2% OV-17 on 80-100 mesh Shimalite W (Shinwakako, Kyoto, Japan); column temperature, 220℃; carrier gas, nitrogen at a flow rate of 35 mL/min.

2.5 Capillary GC/MS conditions

An SPB-50 column (30 m × 0.25 mm i.d., 0.25 μm film thickness) was linked to a Shimadzu GCMS QP2010 mass spectrometer with a computer on-line system. The column temperature was programmed at 180℃ for 2 min isothermally, then to 290℃ at a rate of 3℃/min and held at 290℃ for 2 min. The carrier gas was helium (linear gas velocity, 35 cm/sec; split ratio, 1/25). Electron impact (EI) mass spectra were measured at an ionizing energy at 70 eV by scanning from 50 to 500 *m/z* (0.5 sec/cycle). Chemical ionization (CI) mass spectra were measured at an ionizing energy at 70 eV using isobutane as the reactant gas (gas pressure, 0.08 MPa).

3 Results and Discussion

Fig. 1 shows the gas chromatogram of FAMEs prepared from soybean oil. The FAMEs clearly separated on the SPB-50 column just like when using a medium polar column. We then detached the column from the GC system, attached the column to the GC/MS system, and measured EI and CI mass spectra of five peaks in Fig. 1. Based on fragmentation patterns obtained from the EI mass spectra and the quasi molecular ions obtained from the CI mass spectra (data not shown), the peak assignments in Fig. 1 were determined. This separation of FAMEs on the SPB-50 column was strikingly contrary to what we knew of FAME separation as stated in Section 1.

As shown in Table 1, the equivalent chain-length (ECL) values of methyl 18:1, 18:2 and 18:3 measured on an SE-30 liquid phase[16] are not over 18.00. On non-polar columns FAMEs elute in order of their boiling points (though there are some exceptions). This elution order is reversed in columns with the liquid phases, BDS[14,15] (1,4-butanediol succinate) and DEGS[14,15] (diethyleneglycol succinate), showing more polarity than SE-30 (Table 1), in which unsaturated FAMEs with the same carbon chains separate based on their degrees of unsaturation. In FAME analysis the latter case is now preferred.[6,7]

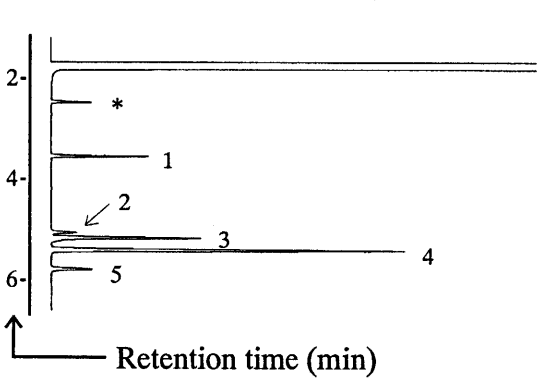

Fig. 1 Capillary gas chromatogram of fatty acid methyl esters prepared from soybean oil.
1=16:0, 2=18:0, 3=18:1, 4=18:2, 5=18:3. Peak assignments were determined by measuring their EI/CI mass spectra on the same column linked to a GC/MS system. Column, SPB-50 column (30 m × 0.25 mm i.d., 0.25 μm film thickness); column temperature, 235℃. BHT (butylated hydroxy toluene) was added to the sample as an antioxidant, and it (*) elutes just after the solvent peak.

We checked the ECL values of the unsaturated FAMEs with C18 carbon chains on a glass column packed with 2% OV-17 liquid phase; this packing material remained in our laboratory for about twenty years. The ECL values of independently injected methyl 18:1, 18:2 and 18:3 were 18.03, 18.04 and 18.24, respectively. The FAMEs prepared from soybean oil could not separate from each other well. We thought this was the reason that using an OV-17 packed-column was unacceptable for research on FAME analysis. Due to the lack of data on FAME analysis with an OV-17 packed-column in previous literature and reviews (R.G. Ackman, private communication), nobody tried to adopt such a column in FAME analysis. Even now nobody uses weak polar FFS capillary columns in FAME analysis.

The supplier of the SPB-50 column informs us that SPB-50 is composed of 50% phenylsiloxane/50% methylsiloxane stationary phase, and that it is equivalent to OV-17, and also that McReynolds' constants[18] of the column is X'=125, Y'=175, Z'=183, U'=268 and S'=220 (sum of X' to S': 971). These constants are the typical ones found in weak polar stationary phases. In medium polar liquid phases (for packed-column use), the sum of McReynolds' constants (X' to S')

Table 1 Equivalent chain length values of C18 fatty acid methyl esters on liquid phases for packed-column use[a]

	Liquid phase (for packed-column use)			
Fatty acid	SE-30	OV-17	BDS	DEGS
18:0	18.00	18.00[b]	18.00	18.00
18:1	17.68	18.03[b]	18.20	18.49
18:2	17.61	18.04[b]	18.78	19.14
18:3	17.66	18.24[b]	19.50	20.06
Column polarity	Non-polar	Weak polar	Medium polar	Polar
McReynolds' constants[c]	217	884	2657	3504

[a] Cited from Refs. 14-16.
[b] Measured in this study. GC conditions are given in the text.
[c] Sum of X' to S' from Ref. 18.

Table 2 Equivalent chain length values of fatty acid methyl esters on weak polar flexible fused-silica capillary columns

	Column name (internal diameter)			
Fatty acid	SPB-50 (0.25 mm)	SPB-50 (0.53 mm)	DB-17MS (0.25 mm)	BPX50[a] (0.25 mm)
18:0	18.00	18.00	18.00	18.00
18:1	18.09	18.10	18.07	18.07
18:2	18.31	18.33	18.27	18.27
18:3	18.63	18.66	18.58	18.59
20:4	20.42	20.43	20.33	20.34
20:5	20.75	20.75	20.65	20.66
22:6	22.86	22.87	22.74	22.75

Each value is an average of two or three determinations.
The column length (30 m), column temperature (isothermally held at 235℃) and the carrier gas (helium, linear gas velocity: 30.0 cm/sec) were the same for all the columns. Other GC conditions are given in the text.
[a] according to the supplier, the chemical composition of the stationary phase is somewhat different from those of SPB-50 and DB-17MS.

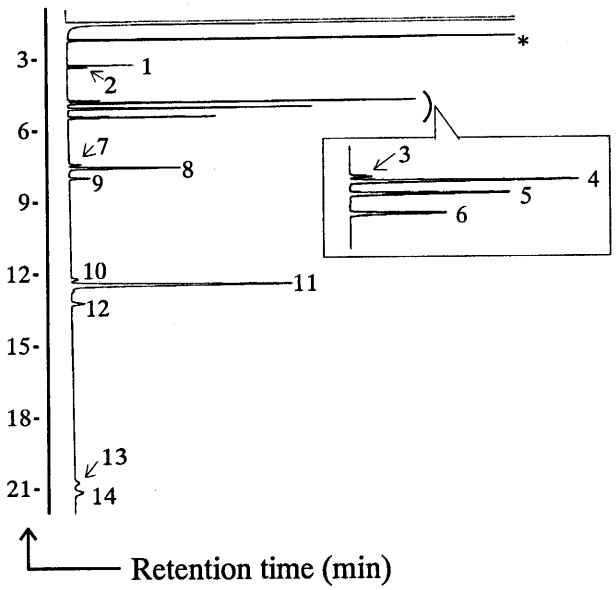

Fig. 2 Capillary gas chromatogram of a standard mixture of fatty acid methyl esters (GLC-421-A, from Nu-Chek Prep).
1=16:0, 2=16:1, 3=18:0, 4=18:1, 5=18:2, 6=18:3, 7=20:0, 8=20:1, 9=20:2, 10=22:0, 11=22:1, 12=22:2, 13=24:0, 14=24:1. Column, SPB-50 column (30 m × 0.25 mm i.d., 0.25 μm film thickness); column temperature, 235℃. BHT (butylated hydroxy toluene) was added to the sample as an antioxidant, and it (*) elutes just after the solvent peak.

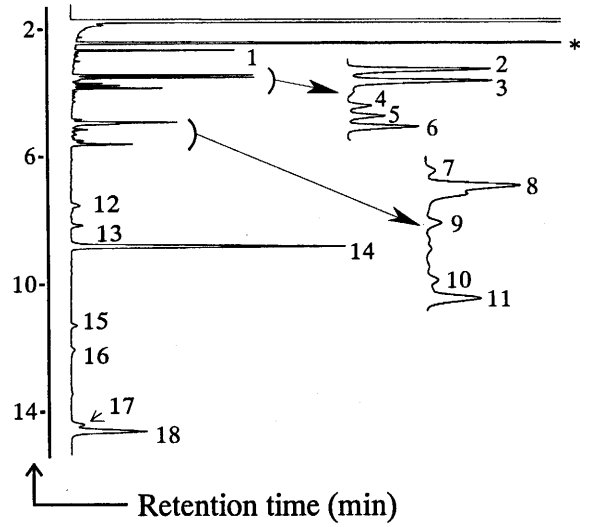

Fig. 3 Capillary gas chromatogram of fatty acid methyl esters prepared from fish oil capsules.
1=14:0, 2=16:0, 3=16:1, 4=16:2, 5=16:3, 6=16:4, 7=18:0, 8=18:1, 9=18:2, 10=18:3, 11=18:4, 12=20:1, 13=20:4, 14=20:5, 15=21:5, 16=22:1, 17=22:5, 18=22:6. Peak assignments were determined by comparing their retention times to those of authentic standards and also by measuring their EI/CI mass spectra on the same column linked to a GC/MS system. Column, SPB-50 column (30 m × 0.25 mm i.d., 0.25 μm film thickness); column temperature, 235℃. BHT (butylated hydroxy toluene) was added to the sample as an antioxidant, and it (*) elutes just after the solvent peak.

was 2657 for a typical polyester phase, BDS (Table 1) and 2424 for a typical polysiloxane phase, SP-2300 (cyanopropyl phenylsiloxane).[15] In polar liquid phases (for packed-column use), the sum of McReynolds' constants (X' to S') was 3504 for a typical polyester phase, DEGS (Table 1). Lipid chemists understand that on non-polar columns the unsaturated C18 FAMEs elute before methyl 18:0, and that on medium polar columns the unsaturated C18 FAMEs elute after methyl 18:0 (and methyl 18:3 elutes before methyl 20:0), and that on more polar columns methyl 18:3 elutes after methyl 20:0 (this is in keeping with the same elution order as that on medium polar columns).

We then tested other FFS capillary columns which the suppliers claim to be equivalent to OV-17 or to SPB-50. Table 2 shows the ECL values of several FAMEs measured on two types of SPB-50 (0.25 and 0.53 mm i.d.) and on two other brand types of DB-17MS and BPX50 under the same operating conditions.

On the four types of columns tested, FAMEs including methyl 18:0, 18:1, 18:2, 18:3, 20:4, 20:5 and 22:6, separated from each other well. This suggests that weak polar FFS capillary columns now commercially available can be used for FAME analysis without any troubles. Furthermore ECL value of methyl 22:6 is below 23.00. This means that even highest unsaturated methyl 22:6 elutes before a saturated ester with one more longer carbon chain, methyl 23:0. Since some odd-chain saturated fatty acids exist in natural lipids, using these weak polar FFS capillary columns in FAME analysis will allow chromatographers to identify peaks easily. At least there is not the "chain-length overlap problem"[19] between main components of FAMEs.

Fig. 2 shows the gas chromatogram of a FAME mixture (GLC-421-A) which is often used for testing column quality. Clear-cut separation is observed. The FAME mixture containing highly unsaturated methyl 20:5 and 22:6 is easily analyzed as shown in Fig. 3. We think that as more and more researchers take advantage of our data presented here, applying weak polar capillary columns to analyze FAMEs prepared from natural lipids or prepared from food samples (quality control in manufacturing) will become popular.

A part of this study was presented at the annual meeting of Japan Oil Chemists' Society held in Yokohama in September, 2005.

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