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Short communication

Gas chromatographic separation of fatty acid methyl esters on weakly polar capillary columns

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Abstract

It was found that weakly polar columns, routinely used in capillary GC for analyzing sterols, food additives, etc., can also be used for separating fatty acid methyl esters (FAMEs). On these columns, FAMEs elute in the order of their unsaturation. The equivalent chain-length value of methyl 22:6 is below 23.00. This means FAMEs within a carbon chain length, having up to six double bonds, elute before the next (one carbon longer) saturated FAME elutes. Peak identification is easy. Weakly polar columns are compatible in both GC and GC/MS systems.

Keywords: Separation of fatty acid methyl esters; Equivalent chain-length value; Capillary gas chromatography; Weakly polar capillary column; Fish oil capsule

1. Introduction

In capillary GC, chromatographers use weakly polar[1] columns to analyze sterols, food additives, etc.; they use medium polar (see footnote 1) or polar (see footnote 1) columns to analyze fatty acid methyl esters (FAMEs) [1–7]. To our knowledge, weakly polar columns are not used for FAME analysis.

While we were using a weakly polar column (SPB-50)[2] for sterol analysis, we injected FAMEs into it just to see what would happen. To our surprise, unsaturated FAMEs within a chain length separately eluted after the corresponding saturated FAME eluted; the elution pattern resembled one that is obtained on a medium polar column.

In this communication, the elution patterns of FAMEs (including FAMEs from fish oil capsules) on six kinds of commercially available weakly polar columns are presented.

2. Materials and methods

2.1. Weakly polar capillary columns

Same-sized narrow bore (30 m × 0.25 mm I.D., 0.25 μm film thickness) and wide bore (30 m × 0.53 mm I.D., 0.50 μm film thickness) columns were used: SPB-50 from Supelco (Bellefonte, PA, USA); BPX50 from SGE International (Ringwood, Australia); DB-17ms and DB-17 from Agilent Technologies (Palo Alto, CA, USA).

2.2. Chemicals

The following authentic standard FAME mixtures were from Nu-Chek Prep (Elysian, MN, USA): GLC-409 (11 kinds of saturated FAMEs); GLC-421-A (14 kinds of saturated and unsaturated FAMEs for checking column quality); 2A (containing equal amounts of methyl 18:0, 18:1, 18:2, 18:3 and 20:4); 3A (containing equal amounts of methyl 18:2, 18:3, 20:4 and 22:6). Authentic standard fatty acids, 13:0, 23:0, 18:1(*trans*-9) and 20:5 (EPA), were from Wako (Osaka, Japan). Soybean oil, lanolin and fish oil capsules (enriched with 20:5 and 22:6) were from

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[1] Technical terms vary when expressing column polarity [4,8–10]. In this communication, it is classified as weakly polar, medium polar and polar: the sum of McReynolds' constants (X', Y', Z', U' and S') [11] is ca. 1000 for weakly polar, 2000–3000 for medium polar and more than 3000 for polar.

[2] The supplier of the SPB-50 column states the following: the sum of McReynolds' constants (X', Y', Z', U' and S') is 971 for this column; and it is equivalent to the OV-17 (50% diphenylsiloxane/50% dimethylsiloxane liquid phase) packed-column. Researchers used the OV-17 packed-column to analyze sterols before the introduction of flexible fused silica capillary columns [1,8,12].

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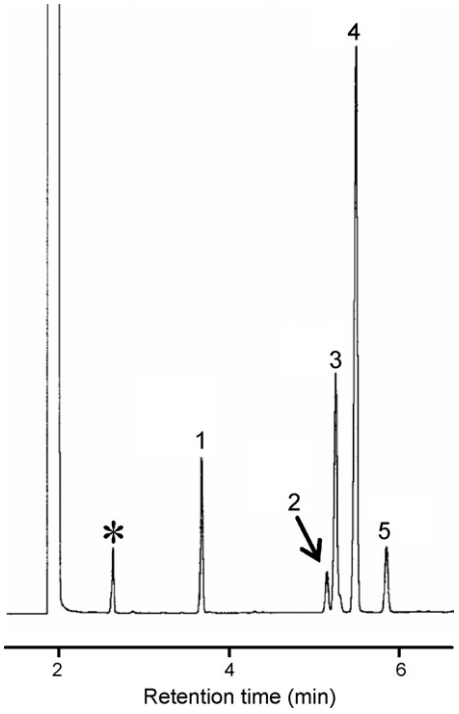

Fig. 1. Capillary gas chromatogram of FAMEs prepared from soybean oil: 1 = 16:0, 2 = 18:0, 3 = 18:1, 4 = 18:2 and 5 = 18:3. Column, SPB-50 (30 m × 0.25 mm I.D., 0.25 μm film thickness); column temperature, 235 °C; carrier gas, helium (linear gas velocity, 30 cm/s; split ratio, 1/30). BHT (butylated hydroxy toluene) was added to the sample as an antioxidant, and it (*) elutes just after the solvent peak. Peaks were identified by comparing the retention times of these FAMEs with those of the authentic standards and also by measuring their EI/CI mass spectra on the same column connected to the GC/MS system.

local markets. Other chemicals were of the highest reagent grade available.

2.3. Preparation of FAMEs

Standard fatty acids were methylated with 14% BF_3/methanol [13]. Soybean oil, lanolin and fish oil capsules were converted to FAMEs by methanolysis with 0.5 M KOH/methanol and successive methylation with 14% BF_3/methanol [13]. All the resulting FAMEs were purified by silica gel TLC [13].

2.4. Capillary GC

A Shimadzu GC-17A gas chromatograph (Kyoto, Japan), with a split/splitless injector, a flame ionization detector and an auto sampler/injector, was used with a Shimadzu workstation on-line system (Class-GC10). Operating conditions are given in the corresponding figures and tables.

2.5. Capillary GC/MS

The SPB-50 column was connected to a Shimadzu GCMS-QP2010 mass spectrometer with a computer on-line system. The column temperatures were the same as those for capillary GC (Figs. 1 and 3). The ion source temperature was 200 °C, and the interface temperature was 280 °C. The carrier gas was helium (linear gas velocity, 35 cm/s; split ratio, 1/25). Electron impact (EI) mass spectra were measured at an ionizing energy of 70 eV by scanning from 50 to 500 *m/z* (0.5 s/cycle). Chemical ionization (CI) mass spectra were measured at an ionizing energy of 70 eV, using isobutane as the reactant gas (gas pressure, 0.08 MPa).

3. Results and discussion

3.1. Analysis of FAMEs on the SPB-50 column with GC

On the weakly polar SPB-50 column, FAMEs prepared from soybean oil separate (Fig. 1) as they would on a medium polar column, except methyl 18:1(11) does not separate from methyl 18:1(9). Fig. 2 shows the gas chromatogram of the authentic standard FAME mixture, GLC-421-A. Methyl 24:1 elutes within 22 min. Also FAMEs prepared from fish oil capsules enriched with 20:5 and 22:6 (operating conditions as in Fig. 1) were analyzed. Methyl 18:1(9) and 18:1(11) poorly separated (chromatogram not shown).

Next, with the FAMEs from the fish oil capsule, testing the limits of the column by changing the operating conditions was continued. Fig. 3 illustrates one of the chromatograms from an analysis in which column temperature programming was employed. Separation of methyl 18:1(9) and 18:1(11) slightly improves. Baseline separations of double-bond positional isomers, of saturated and monounsaturated FAMEs, and of methyl 22:5 and 22:6 cannot be achieved (Fig. 3). Furthermore, separation of double-bond geometrical isomers was impossible (chromatogram not shown).

Although weakly polar columns afford poor resolution of these components, they can be used for FAME analysis as long as their limitations are understood.

3.2. Analysis of FAMEs on the SPB-50 column with GC/MS

To identify the FAMEs, the peak retention times of the FAME samples (Figs. 1 and 3) were compared with those of the authentic standards, and the EI/CI mass spectra (data not shown) on the same SPB-50 column connected to the GC/MS system were measured. The column temperatures at 235 °C (Fig. 1) and 280 °C (Fig. 3, final temperature) were lower than the maximum operating temperature (310 °C, according to the supplier). The mass spectra were clear because of low column bleeding.

3.3. Equivalent chain-length (ECL) values of FAMEs on weakly polar columns

The authentic standard FAME mixtures [2A and 3A (cf., Section 2.2)] and the authentic standard methyl 20:5 were analyzed on six kinds of weakly polar columns. Then the ECL values [3] were calculated (Table 1).

On each column the ECL value of methyl 22:6 is below 23:00. This means that the elution of the following FAMEs is straight forward: peaks of methyl 22:1, 22:2, 22:3, 22:4, 22:5 and 22:6 appear, in that order, between the peaks of methyl 22:0 and 23:0.