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Bulletin 907

Comparison of 37 Component FAME Standard on Four Capillary GC Columns

The fatty acid composition of an average diet is a complex mixture of saturated, monounsaturated, and polyunsaturated fatty acids with a variety of carbon chain lengths. To confirm the identification of key fatty acids, several different standards and capillary columns are required. The Supelco 37 Component FAME Mix can be used to identify key FAMEs in many food products. Here, this mix is analyzed on four capillary columns of different polarities: Omegawax 250, PAG, SP-2380, and SP-2560, to compare elution patterns of the key FAMEs. A polyethylene glycol and cyanosilicone column combination provide the ideal tool for best confirmational analyses of FAMEs.

Key Words
- fatty acids • fatty acid methyl esters • FAMEs
- polyunsaturates • *trans* fatty acids

With the implementation of the Nutrition Labeling and Education Act of 1990 by the U.S. Food and Drug Administration (FDA), the total fat and saturated fat contents of a food must be listed on its label. An optional listing of the *cis*-monounsaturated and *cis,cis*-methylene interrupted polyunsaturated fatty acids can be stated on the label as "monounsaturated" and "polyunsaturated" fatty acids. Unfortunately for the food analyst, determining the fatty acid composition is difficult because a food can contain many different fatty acids of various carbon chain lengths. For example, milk contains fatty acids ranging from butyric acid (C4:0) to arachidic acid (C20:0). Included in the fatty acid composition is a homologous series of even-carbon-numbered saturated fatty acids, monoenoic, hexadecenoic, and octadecenoic fatty acids, and polyunsaturated C18 dienoic and trienoic fatty acids. Milk and butter are also known to contain small amounts of *trans* fatty acids.

Some vegetable oils, included in many diets as a part of the food processing or preparation step, contain fatty acids ranging from C6 to C24 in carbon number, with varied amounts of saturated and unsaturated *cis* fatty acids. If the oils have been hydrogenated, as in margarine, *trans* fatty acids also will be present. In addition, meat and fish contribute saturated, monounsaturated, and *cis,cis*-methylene interrupted polyunsaturated fatty acids to the diet.

Fatty acids are typically analyzed as methyl esters, using capillary GC. Multiple standards and capillary columns can be required to identify key fatty acids from complex food samples, inefficiently expending laboratory time and money. The Supelco™ 37 Component FAME Mix can be used to identify key fatty acid methyl esters (FAMEs) in many foods. This mix contains FAMEs ranging from C4:0 to C24:1, including most of the important saturated, monounsaturated, and polyunsaturated FAMEs (Table 1).

The Supelco 37 Component FAME Mix is designed to mimic the fatty acid composition of many food samples. For example, the saturated series of fatty acids starting with C4, along with the unsaturated and polyunsaturated C16 and C18 FAMEs, mimic milk fat. Representative components in hydrogenated and nonhydrogenated vegetable oils are a homologous series of even-carbon-numbered saturated FAMEs from C8 through C24, the monounsaturated C16:1, *cis* and *trans* C18:1, C20:1, C22:1, and the polyunsaturated octadecenoic FAMEs.

Table 1. Composition of Supelco 37 Component FAME Mix

Peak ID■	Component (acid methyl esters)	Weight (%)
1	C4:0 (Butyric)	4
2	C6:0 (Caproic)	4
3	C8:0 (Caprylic)	4
4	C10:0 (Capric)	4
5	C11:0 (Undecanoic)	2
6	C12:0 (Lauric)	4
7	C13:0 (Tridecanoic)	2
8	C14:0 (Myristic)	4
9	C14:1 (Myristoleic)	2
10	C15:0 (Pentadecanoic)	2
11	C15:1 (*cis*-10-Pentadecenoic)	2
12	C16:0 (Palmitic)	6
13	C16:1 (Palmitoleic)	2
14	C17:0 (Heptadecanoic)	2
15	C17:1 (*cis*-10-Heptadecenoic)	2
16	C18:0 (Stearic)	4
17	C18:1n9c (Oleic)	4
18	C18:1n9t (Elaidic)	2
19	C18:2n6c (Linoleic)	2
20	C18:2n6t (Linolelaidic)	2
21	C18:3n6 (γ-Linolenic)	2
22	C18:3n3 (α-Linolenic)	2
23	C20:0 (Arachidic)	4
24	C20:1n9 (*cis*-11-Eicosenoic)	2
25	C20:2 (*cis*-11,14-Eicosadienoic)	2
26	C20:3n6 (*cis*-8,11,14-Eicosatrienoic)	2
27	C20:3n3 (*cis*-11,14,17-Eicosatrienoic)	2
28	C20:4n6 (Arachidonic)	2
29	C20:5n3 (*cis*-5,8,11,14,17-Eicosapentaenoic)	2
30	C21:0 (Henicosanoic)	2
31	C22:0 (Behenic)	4
32	C22:1n9 (Erucic)	2
33	C22:2 (*cis*-13,16-Docosadienoic)	2
34	C22:6n3 (*cis*-4,7,10,13,16,19-Docosahexaenoic)	2
35	C23:0 (Tricosanoic)	2
36	C24:0 (Lignoceric)	4
37	C24:1n9 (Nervonic)	2

■ Peak numbers in Figures A–D.

FAMEs typically found in fish and meat include the previously mentioned saturated and unsaturated FAME isomers, and the key omega-3 and omega-6 polyunsaturated FAMEs. Fish contain large amounts of the omega-3 fatty acids, such as C20:5n3 and C22:6n3, whereas meats are richer in the omega-6 fatty acids, such as arachidonic acid (C20:4n6). All of the omega-3 and omega-6 fatty acids are *cis, cis* methylene interrupted FAMEs. Also included in the 37 component mix are some of the odd-carbon-numbered FAMEs that are found in some foods.

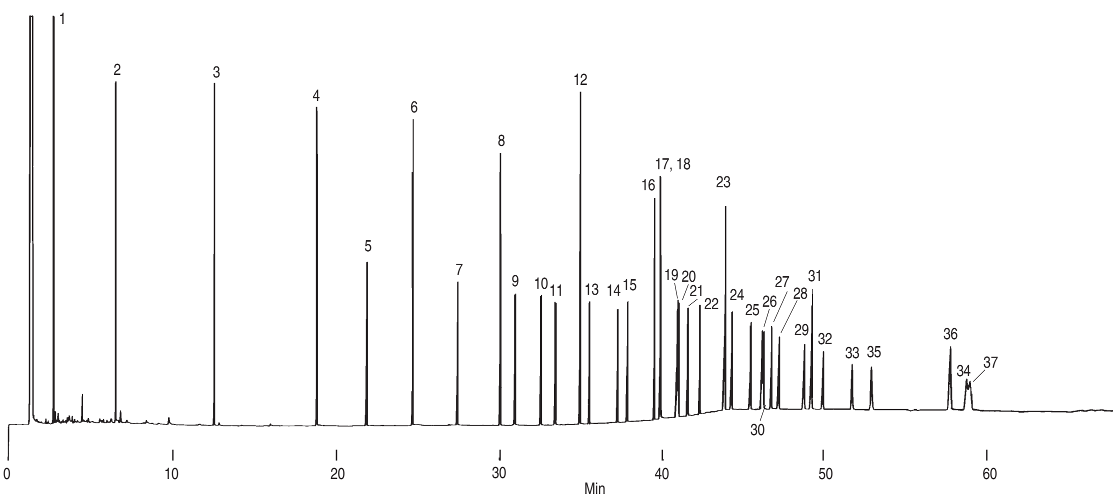
Analysts evaluating food samples must choose from a number of capillary columns. The sample type and the information being sought determines the proper column choice. Routinely, polyethylene glycol-based capillary columns are used for FAME analysis of marine fish oils and meat samples, as they will elute the FAME isomers according to carbon chain length and degree of unsaturation. Cyanosilicone capillary columns are widely used for analyzing vegetable oils, as they provide resolution of *cis* and *trans* FAMEs. However, some of the carbon chain lengths usually overlap on cyanosilicone phases, causing problems in peak identification.

To compare columns for analyzing FAMEs, we evaluated the Supelco 37 Component FAME Mix on four capillary columns of different polarities: Omegawax™ 250, PAG, SP™-2380, and SP-2560 (Figures A-D). Omegawax 250, a bonded polyethylene glycol-based phase, is used for analyses of polyunsaturated marine fish oils. For comparison, a phase of similar polarity, the slightly less polar polyalkylene glycol (PAG) phase, was evaluated. This phase contains a percentage of propylene oxide in its polymer backbone, rather than 100% polyethylene oxide (Omegawax 250). Two high-polarity cyanosilicone columns were evaluated: SP-2380 (95% cyanopropyl 5% phenyl polysiloxane) and SP-2560 (100% bis-cyanopropyl polysiloxane).

Injection and detection parameters for the analyses were identical, except for oven temperature and carrier gas linear velocity (Table 2).

To evaluate the figures, we compared the two lower polarity columns, then compared these results with observations for the higher polarity columns. The Omegawax 250 and PAG column analyses (Figures A and B) show slight differences in the elution

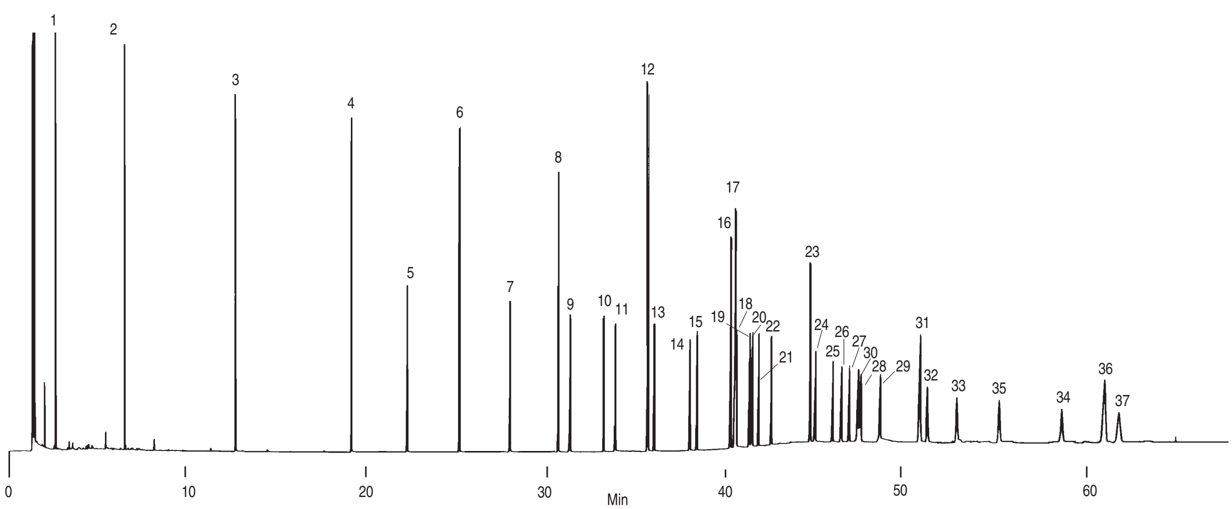
Figure A. Supelco 37 Component FAME Mix on Omegawax 250 Column●



● See Table 1 for peak IDs and Table 2 for conditions.

794-0661

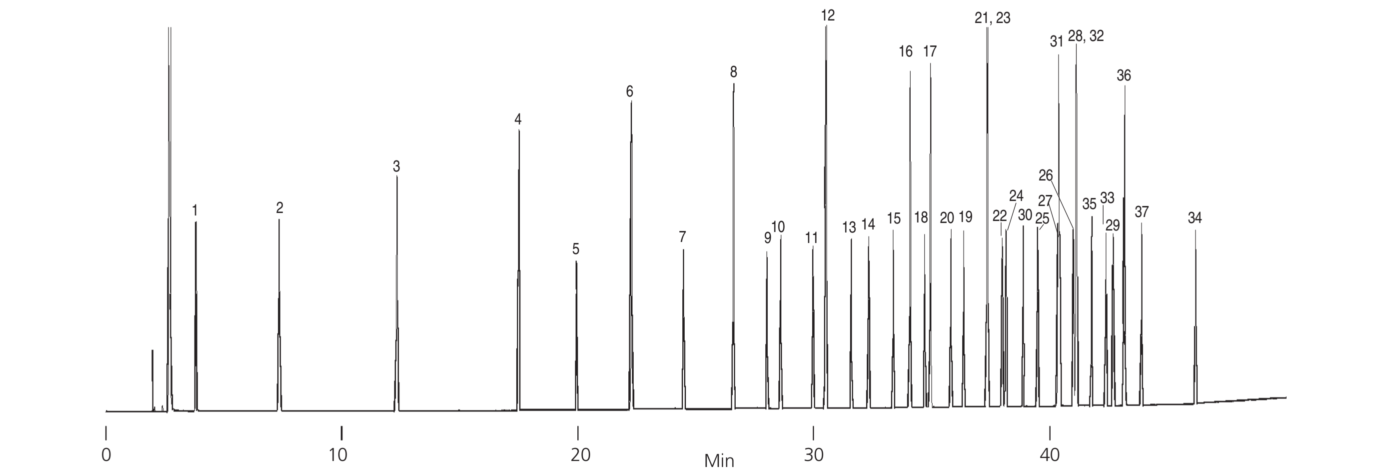
Figure B. Supelco 37 Component FAME Mix on PAG Column●



● See Table 1 for peak IDs and Table 2 for conditions.

794-0660

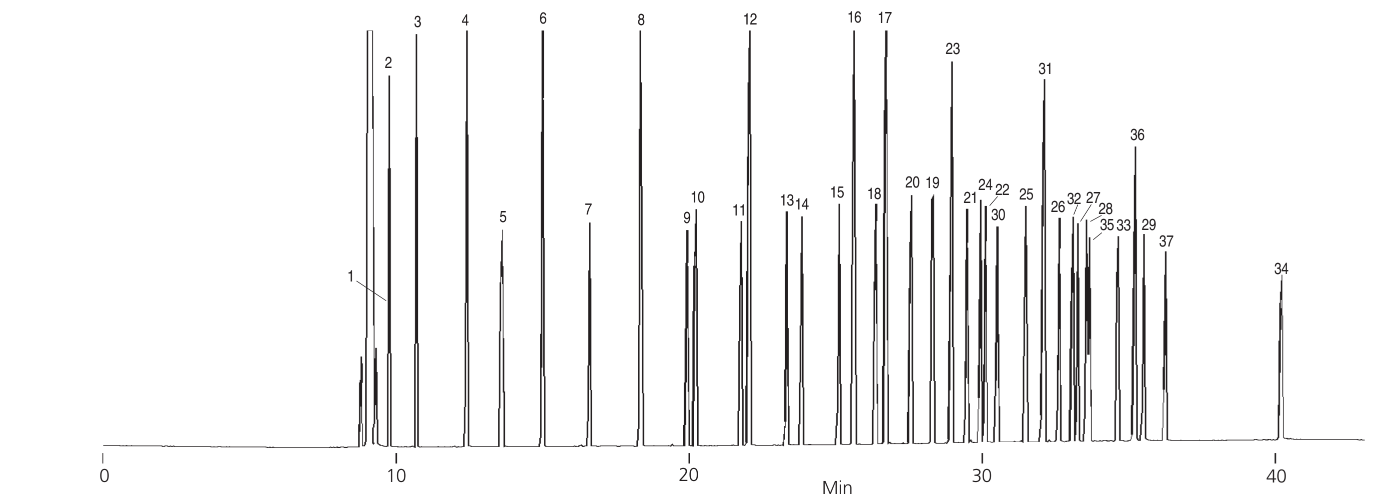
Figure C. Supelco 37 Component FAME Mix on SP-2380 Column•



ǀSee Table 1 for peak IDs and Table 2 for conditions.

795-0781

Figure D. Supelco 37 Component FAME Mix on SP-2560 Column•



ǀ See Table 1 for peak IDs and Table 2 for conditions.

795-0472

patterns for the polyunsaturated FAMEs. The most notable differences occur with the more highly unsaturated longer chain length FAMEs. The less polar PAG column provides a truer carbon chain length separation. The even-carbon-numbered FAMEs elute strictly according to carbon number and degree of unsaturation. For the Omegawax 250 column, there is some overlap, as C22:6n3 elutes after C24:0.

The higher-polarity SP-2380 and SP-2560 columns show a much different elution pattern. An advantage is that the cyanosilicone phases resolve *cis* and *trans* FAMEs, with the *trans* isomer eluting prior to the *cis* isomer. The SP-2560 column, in particular, is designed to resolve positional geometric FAME isomers. The resolution and elution patterns for the C18:1n9t and C18:1n9c and C18:2n6t and C18:2n6c isomers on all four columns show the differences in selectivity and resolving power. The polyethylene glycol columns offer slight resolution of the *cis* and *trans* isomers, with the *cis* isomer eluting first.

A disadvantage of using the high polarity cyanosilicone columns is that there is significant carbon chain overlap in the elution patterns. Most of the trienoic and more polyunsaturated FAMEs elute after the next even-carbon-numbered saturated FAME. For example, C18:3n6 and C18:3n3 elute after the saturated C20 FAME. This can lead to peak identification problems. The polyalkylene glycol columns produce an elution pattern that more closely follows carbon chain length, and the higher polarity cyanosilicone columns produce better *cis* and *trans* FAMEs resolutions.

Given the differences in elution patterns of FAME isomers on the four columns, we feel that using both a polyethylene glycol column and a cyanosilicone column with the Supelco 37 Component FAME Mix provides the ideal tool for confirmation analyses of FAMEs. Columns of equal dimensions can be connected to a single injection port and separate detectors and, by making a single injection, two separate elution profiles will be generated.

Table 2. Conditions for Analyzing the Supelco 37 Component FAME Mix on Omegawax 250, PAG, SP-2380, and SP-2560 Columns

General Conditions

det.:	FID (2 x 10^{-11}), 260 °C
sample concentration:	10 mg/mL
inj.:	1 µL of Supelco 37 Component FAME Mix, split 100:1, 250 °C

Column-Specific Conditions

column:	Omegawax 250, 30 m x 0.25 mm I.D. x 0.25 µm film (2-4136)
oven:	50 °C (2 min.) to 220 °C at 4 °C/min., hold 15 min.
carrier gas:	helium, 30 cm/sec., 205 °C
column:	PAG, 30 m x 0.25 mm I.D. x 0.25 µm film (2-4223)
oven:	50 °C (2 min.) to 220 °C at 4 °C/min., hold 15 min.
carrier gas:	helium, 30 cm/sec., 205 °C
column:	SP-2380, 30 m x 0.25 mm I.D. x 0.20 µm film (2-4110)
oven:	50 °C (2 min.) to 250 °C at 4 °C/min., hold 15 min.
carrier gas:	helium, 20 cm/sec., 150 °C
column:	SP-2560, 100 m x 0.25 mm I.D. x 0.20 µm film▲ (2-4056)
oven:	140 °C (5 min.) to 240 °C at 4 °C/min., hold 15 min.
carrier gas:	helium, 20 cm/sec., 175 °C

Ordering Information:

Description	Cat. No.
Fused Silica Capillary Columns	
Omegawax 250, 30 m x 0.25 mm I.D. x 0.25 µm film	**24136**
PAG, 30 m x 0.25 mm I.D. x 0.25 µm film	**24223**
SP-2380, 30 m x 0.25 mm I.D. x 0.20 µm film	**24110-U**
SP-2560, 100 m x 0.25 mm I.D. x 0.20 µm film▲	**24056**
37 Component FAME Mix, 1 mL, 10 mg/mL FAMEs in methylene chloride.	**47885-U**

For many additional fatty acid standards, refer to the current Supelco catalog.

▲The SP-2560 column is available only in a length of 100 m.

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