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Comparison of New 37 Component FAME Standard on Four Capillary Columns of Different Polarities

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The fatty acid composition of an average person's diet is very complex. One food can contain 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 newly developed, broad-based Supelco 37 Component FAME Mix can aid in identifying key FAMEs in many food products. 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 analysis of FAMEs.

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 as "monounsaturated" and "polyunsaturated" fatty acids. Unfortunately for the food analyst, determining 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 C16 and C18, 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, then *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 average diet.

Fatty acids are typically analyzed as methyl esters, using capillary gas chromatography (GC). Many different standards and capillary columns are required to identify key fatty acids from complex food samples, which inefficiently expend laboratory time and money. The recently developed Supelco™ 37 Component FAME Mix helps identify key fatty acid methyl esters (FAMEs) in many foods. This new mix contains FAMEs ranging in carbon number from C4 to C24:1, including most of the important saturated, monounsaturated, and polyunsaturated FAMEs. Refer to Table 1 for a complete listing.

The Supelco 37 Component FAME Mix is designed to mimic the fatty acid composition of a number of food samples. For example, the saturated series of fatty acids starting with C4, along with the unsaturated and polyunsaturated C16 and C18 FAMEs, will mimic a milk fat sample. Representative components in hydrogenated and pure 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 IDs[■]	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

■ These numbers reference the peaks in Figures A through D.

FAMEs typically found in fish and meat samples include the previously discussed saturated and unsaturated FAME isomers, and the key omega-3 and omega-6 polyunsaturated FAMEs. Fish samples contain large amounts of the omega-3 fatty acids, such as C20:5n3 and C22:6n3, whereas meat samples 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 this new mix are some of the odd-carbon-numbered FAMEs that are found in some food samples.

Analysts must choose from a number of capillary columns to analyze food samples. 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 because they provide resolution of *cis* and *trans* FAMEs. However, the cyanosilicone phases usually overlap some of the carbon chain lengths, which can cause problems in peak identification.

Figure A. Supelco 37 Component FAME Mix on Omegawax 250 Column●
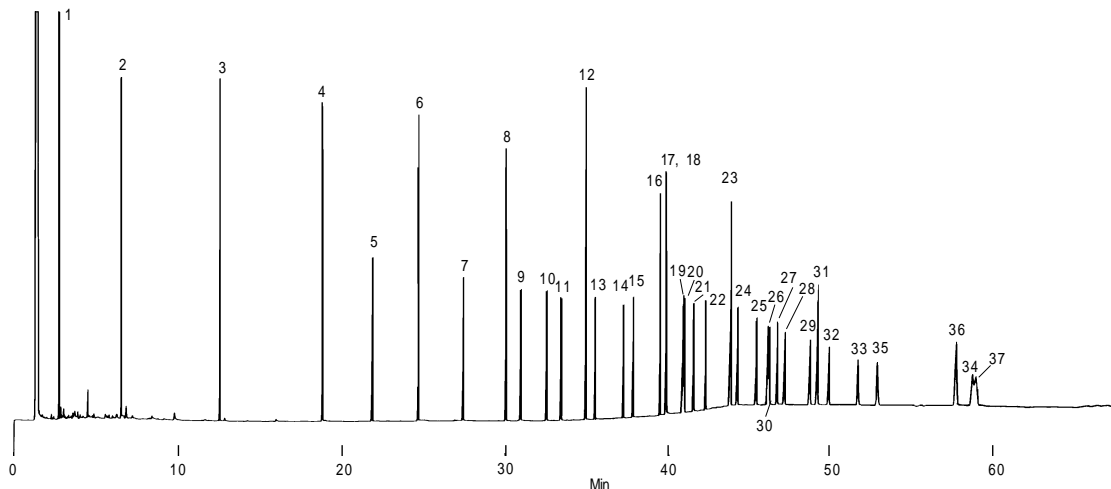
● See Table 1 for peak IDs and Table 2 for conditions.

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•

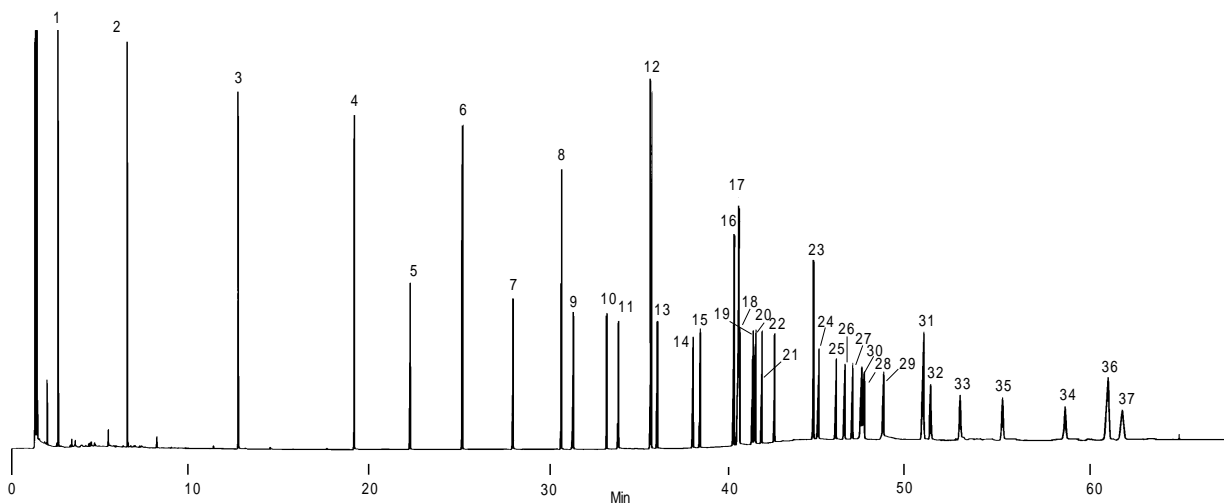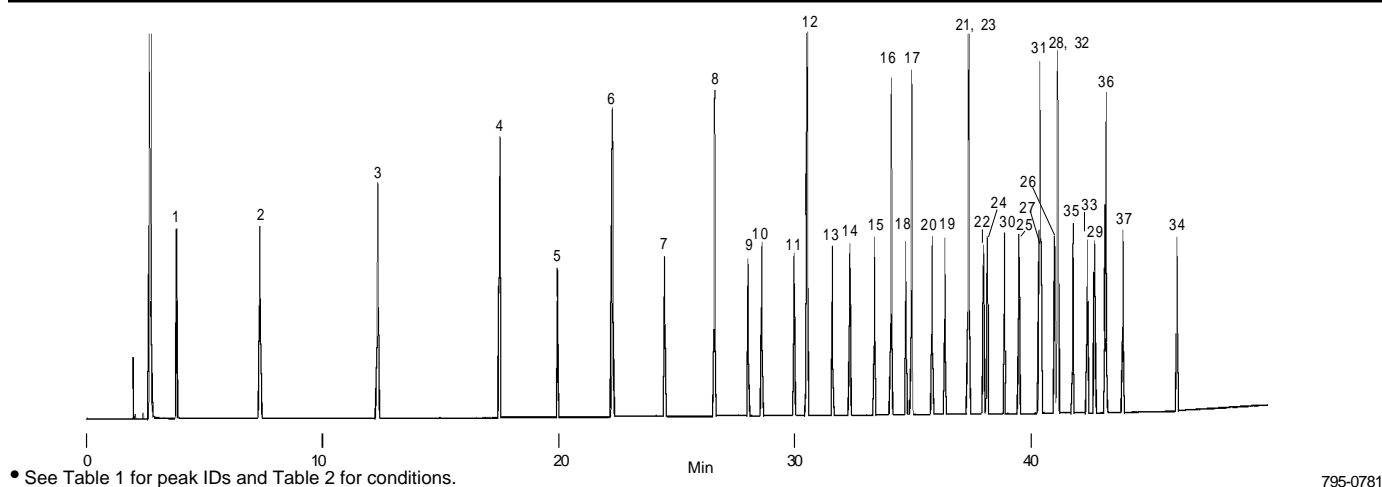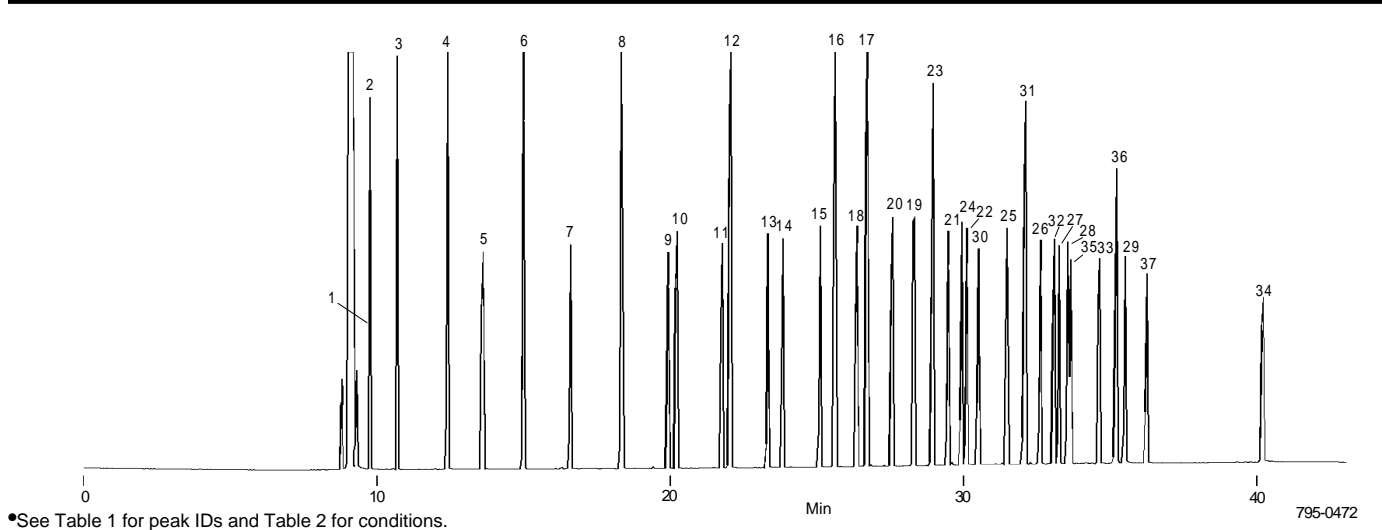• See Table 1 for peak IDs and Table 2 for conditions.   795-0781

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

•See Table 1 for peak IDs and Table 2 for conditions.   795-0472

To compare columns for analyzing FAMEs, we evaluated the Supelco 37 Component FAME Mix on four capillary columns of different polarities: the Omegawax™ 250, the PAG, the SP™-2380, and the SP-2560 (see Figures A thru D). The Omegawax 250 column is a bonded polyethylene glycol-based phase. For comparison with 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% of polyethylene oxide like the Omegawax 250 column. Two high-polarity cyanosilicone columns were evaluated for different elution patterns: SP-2380 (95% cyanopropyl 5% phenyl polysiloxane) and SP-2560 (100% bis-cyanopropyl polysiloxane).

Most of the injection and detection parameters for the analyses were identical, except for oven temperature, and carrier gas linear velocity and set temperature. All analyses were temperature

programmed runs. Refer to Table 2 on the following page for conditions on all four columns.

To evaluate the figures, we will compare the two lower polarity columns and then contrast these results with the two higher polarity columns. The Omegawax 250 and the PAG column analyses (Figures A and B) show slight differences in the elution patterns for the polyunsaturated FAMEs. The most notable differences in the elution patterns occur with the more highly unsaturated longer-chain-length FAMEs. The comparison of these analyses indicates that the less polar PAG column provides a truer carbon chain length separation. All of the even-carbon-numbered FAMEs elute according to carbon number and degree of unsaturation prior to the next saturated even-carbon-numbered FAME eluting. For the Omegawax 250 column, there is some carbon chain overlap, as the C22:6n3 FAME elutes after the C24:0 FAME.

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:	10mg/mL
Inj.:	1µL of Supelco 37 Component FAME Mix, split 100:1, 250°C

Column-Specific Conditions

Column:	**Omegawax 250**, 30m x 0.25mm ID x 0.25µm film
Cat. No.:	**24136**
Oven:	50°C (2 min) to 220°C at 4°C/min, hold 15 min
Carrier:	helium, 30cm/sec, 205°C
Column:	**PAG**, 30m x 0.25mm ID x 0.25µm film
Cat. No.:	**24223**
Oven:	50°C (2 min) to 220°C at 4°C/min, hold 15 min
Carrier:	helium, 30cm/sec, 205°C
Column:	**SP-2380**, 30m x 0.25mm ID x 0.20µm film
Cat. No.:	**24110-U**
Oven:	50°C (2 min) to 250°C at 4°C/min, hold 15 min
Carrier:	helium, 20cm/sec, 150°C
Column:	**SP-2560**, 100m x 0.25mm ID x 0.20µm film
Cat. No.:	**24056**
Oven:	140°C (5 min) to 240°C at 4°C/min, hold 15 min
Carrier:	helium, 20cm/sec, 175°C

port and separate detectors.[▲] By making a single injection, two separate elution profiles will be generated, providing an extensive FAME analysis.

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. By using the Supelco 37 Component FAME Mix with these columns, the key FAMES of many foods can be easily and efficiently identified, thereby eliminating many of the hassles associated with food analyses.

Ordering Information:

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

The higher-polarity SP-2380 and SP-2560 columns show a much different elution pattern. An advantage is that the cyanosilicone phases offer the capability to 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 isomers and the C18:2n6t and C18:2n6c isomers on all four column types show the differences in selectivity and resolving power of the columns. The polyethylene glycol columns do offer slight resolution of the *cis* and *trans* isomers with the *cis* isomer eluting first.

A disadvantage to using the high polarity cyanosilicone columns is that there is a significant amount of carbon chain overlap in the elution patterns. Most of the trienoic and more polyunsaturated FAMEs tend to elute after the next even-carbon-numbered saturated FAME. For example, C18:3n6 and C18:3n3 elute after the saturated C20 FAME, leading to possible peak identification problems.

Given the differences in elution patterns of FAME isomers on the four analyzed columns of different polarities, we found that using both a polyethylene glycol and a cyanosilicone column with the Supelco 37 Component FAME Mix provides the ideal tool for confirmation analyses of FAME samples. When columns of equal dimensions are used, they can be connected to a single injection

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

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

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Fused silica columns manufactured under HP US Pat. No. 4,293,415.

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