Fatty Acid/FAME Application Guide

Analysis of Foods for Nutritional Needs

SUPELCO® Analytical

Free Fatty Acid Analysis

FAME Preparation

FAME Analysis

Fatty Acid / FAME Application Guide

Analysis of Foods for Nutritional Needs

We are cognizant of the impact our products play in nearly every aspect of modern life, from protection of the environment to the safety of consumer products in all market categories. However, it is rewarding when our products can be directly applied to topics of great interest to the general population. One area currently of public interest is nutrition. Obesity, diabetes, and cardiovascular disease, along with their related costs, are increasing in America, Europe, and in other parts of the world. Although heredity contributes, a clear link between diet and these maladies has been firmly established. (1-4)

One measure of the nutritional and health value of a food is its fat content. It is not only total fat, but also the type of fat that must be considered. Some 'good fats' are required for biochemical processes or necessary for dissolving fat-soluble vitamins. Other 'bad fats' interfere with biochemical processes or accumulate in the cardiovascular system, potentially leading to health problems. Currently, there is an increase in research into the safety and health effects of fatty acids and toward understanding their fundamental biochemistry.

For the food chemist, determining the fatty acid composition of a product may be difficult because foods can contain a complex mixture of saturated, monounsaturated, and polyunsaturated fatty acids, each with a variety of carbon chain lengths.

This brochure was assembled to provide food chemists with a valuable resource to assist in identifying the proper products for the GC analysis of fatty acids, either as free fatty acids or as fatty acid methyl esters. Many of these specialized products, such as GC columns, SPE tubes, reagents, and chemical standards, were specifically developed for use in the qualitative and quantitative identification of fatty acids. Details of each of these products are included throughout this brochure, which is arranged by analytical application. The diverse analytical applications, chromatograms, and product listings that are attached within this brochure were selected with the chromatographer in mind, to help them ensure accurate and reproducible analyses.

Want additional information beyond what this brochure provides? Page 23 lists product literature and also recommended reading written by experts and researchers. Another resource is the Sigma-Aldrich/Supelco FAME web site: *sigma-aldrich.com/fame*, where product listings, technical literature detailing how to use these products, chromatograms with peak IDs and conditions, and peer-reviewed literature references can be easily found. Supelco Technical Service chemists are also invaluable sources for providing guidance with the selection and use of applicable products. Supelco Technical Service chemists can be reached at 800-359-3041 (US and Canada only), 814-359-3041, or at *techservice@sial.com*

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Free Fatty Acids

Short chain, volatile fatty acids are typically analyzed in the free form using specialized columns. This group of compounds may be referred to as free fatty acids (FFAs), volatile fatty acids (VFA), or carboxylic acids. The analysis of fatty acids in the free form instead of as fatty acid methyl esters results in easier and quicker sample preparation. Additionally, artifact formation that may result from a derivatization procedure, is eliminated.

This section (pages 3-4) focuses on the analysis of free fatty acids. Details on the preparation (pages 5-6) and analysis (pages 7-20) of fatty acid methyl esters can be found in other sections.

GC Column Choices

For the GC analysis of free fatty acids, a specialized column that will not allow the adsorption of active carboxyl groups is required. The Nukol, with its acidic characteristic, is well-suited for this application, allowing chromatography with excellent peak shapes. For application, USP code, polymer, and temperature limit information, as well as catalog numbers, please refer to page 21.

Chromatograms

The following selected chromatograms for this application are presented to assist the chromatographer in establishing analytical conditions. For assistance, contact Supelco Technical Service at 800-359-3041 (US and Canada only), 814-359-3041, or at *techservice@sial.com*

Figure 1. Short Chain Free Fatty Acids on the Nukol

column: Nukol, 30 m x 0.25 mm I.D., 0.25 µm (24107)
oven: 185 °C
det.: FID
carrier gas: helium, 20 cm/sec
injection: 1 µL, 100:1 split
sample: Volatile Free Acid Mix (46975-U), each analyte at 10 mM in deionized water

1. Acetic acid
2. Propionic acid
3. Isobutyric acid
4. Butyric acid
5. Isovaleric acid
6. Valeric acid
7. Isocaproic acid
8. Caproic acid
9. Heptanoic acid

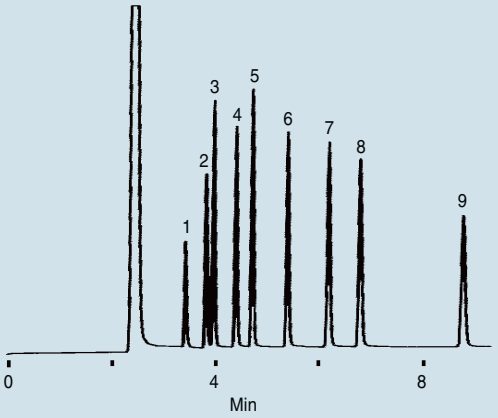

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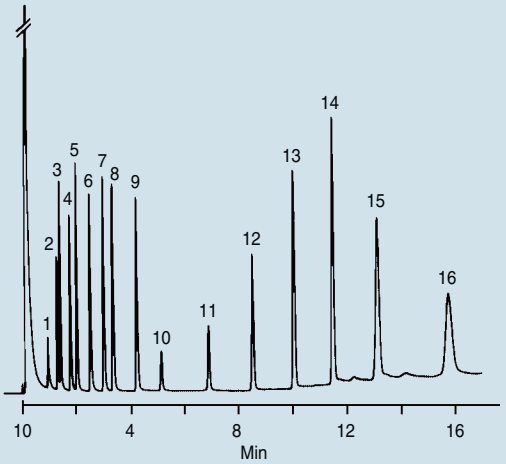
Figure 2. Short and Long Chain Free Fatty Acids on the Nukol

column: Nukol, 15 m x 0.53 mm I.D., 0.50 µm (25326)
oven: 100 °C, 10 °C/min. to 220 °C
det.: FID
carrier gas: helium, 30 mL/min.
injection: 0.5 µL, direct injection
sample: 16 analytes, at various concentrations from 50 to 800 µg/mL

1. Acetic acid
2. Propionic acid
3. Isobutyric acid
4. Butyric acid
5. Isovaleric acid
6. Valeric acid
7. Isocaproic acid
8. Caproic acid
9. Heptanoic acid
10. Octanoic acid
11. Decanoic acid
12. Dodecanoic acid
13. Tetradecanoic acid
14. Hexadecanoic acid
15. Octadecanoic acid
16. Eicosanoic acid



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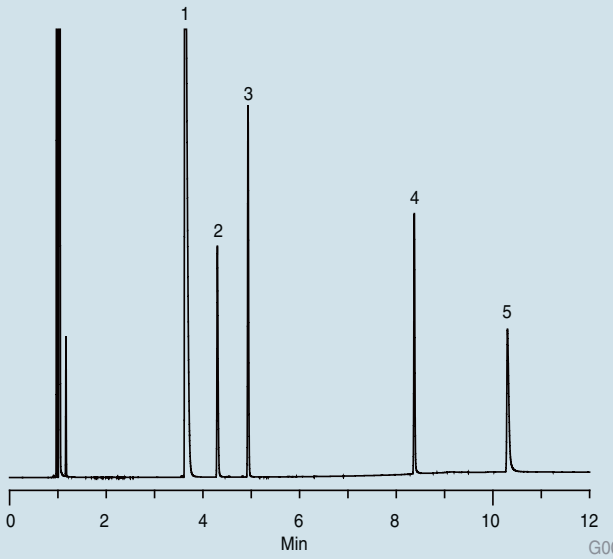
Free Fatty Acids

Figure 3. Organic Acids on the Nukol

column: Nukol, 15 m x 0.32 mm I.D., 0.25 µm (24130)
oven: 80 °C (1 min.), 15 °C/min. to 200 °C (3 min.)
inj.: 250 °C
det.: FID, 250 °C
carrier gas: helium, 2 mL/min. constant
injection: 1 µL, 100:1 split
liner: 4 mm I.D., split, cup design
sample: 5 analytes, at concentrations indicated in 1 M H_3PO_4

1. Acetic acid, 8%
2. Propionic acid, 0.7%
3. Butyric acid, 0.7%
4. Sorbic acid, 0.7%
5. Benzoic acid, 0.7%



Chemical Standards

Standards for the determination of free fatty acids should be purchased from a chemical manufacturer with knowledge in the preparation, handling, storage, and shipment of volatile analytes. Sigma-Aldrich, with over 40 years in chemical standard manufacturing through the Supelco brand, offers the following standards.

Description			Cat. No.
Water Soluble Fatty Acid Mix 2 (WSFA-2) Each analyte at 0.1 wt. % in deionized water, 5 mL			47056
Acetic acid	*Isobutyric acid*	*Propionic acid*	
Butyric acid	*Isovaleric acid*	*Valeric acid*	
Water Soluble Fatty Acid Mix 4 (WSFA-4) Each analyte at 0.1 wt. % in deionized water, 5 mL			47058
Acetic acid	*Isovaleric acid*	*Propionic acid*	
Butyric acid	*2-Methylbutyric acid*	*Valeric acid*	
Isobutyric acid			
Volatile Free Acid Mix Each analyte at 10 mM in deionized water, 100 mL			46975-U
Acetic acid	*Hexanoic acid*	*4-Methylvaleric acid*	
Butyric acid	*Isobutyric acid*	*Propionic acid*	
Formic acid	*Isovaleric acid*	*Valeric acid*	
Heptanoic acid			
Non-Volatile Acid Standard Mix Each analyte at 0.01 meq/mL in deionized water, 100 mL			46985-U
Fumaric acid	*Methylmalonic acid*	*Pyruvic acid*	
Lactic acid	*Oxalacetic acid*	*Succinic acid*	
Malonic acid	*Oxalic acid*		

Solvents

All CHROMASOLV® solvents are prepared with unsurpassed attention to quality, and are designed for meeting stringent purity standards.

Description	Pkg. Size	Cat. No.
Chloroform, >=99.8%,amylene stabilized	100 mL 1 L	34854-100ML 34854-1L
Dichloromethane, >=99.8%, amylene stabilized	100 mL 1 L	34856-100ML 34856-1L
Hexane, >=95%	100 mL 1 L	270504-100ML 270504-1L
Heptane, >=99%	100 mL 1 L	34873-100ML 34873-1L
Toluene, 99.9%	100 mL 1 L	34866-100ML 34866-1L

Fatty Acid Methyl Ester (FAME) Preparation

GC can be used to analyze fatty acids either as free fatty acids or as fatty acid methyl esters. Details on the analysis of free fatty acids can be found on pages 3-4.

The primary reasons to analyze fatty acids as fatty acid methyl esters include:

- In their free, underivatized form, fatty acids may be difficult to analyze because these highly polar compounds tend to form hydrogen bonds, leading to adsorption issues. Reducing their polarity may make them more amenable for analysis.
- To distinguish between the very slight differences exhibited by unsaturated fatty acids, the polar carboxyl functional groups must first be neutralized. This then allows column chemistry to perform separations by boiling point elution (pages 7-9), and also by degree of unsaturation (pages 10-12), position of unsaturation (pages 13-15), and even the cis vs. trans configuration of unsaturation (pages 16-20).

The esterification of fatty acids to fatty acid methyl esters is performed using an alkylation derivatization reagent. Methyl esters offer excellent stability, and provide quick and quantitative samples for GC analysis. As shown in Figure 4, the esterification reaction involves the condensation of the carboxyl group of an acid and the hydroxyl group of an alcohol. Esterification is best done in the presence of a catalyst (such as boron trichloride). The catalyst protonates an oxygen atom of the carboxyl group, making the acid much more reactive. An alcohol then combines with the protonated acid to yield an ester with the loss of water. The catalyst is removed with the water. The alcohol that is used determines the alkyl chain length of the resulting esters (the use of methanol will result in the formation of methyl esters whereas the use of ethanol will result in ethyl esters).

Figure 4. Esterification Reaction

$$R\text{-}COOH + CH_3OH \xrightarrow{BCl_3} R\text{-}COO\text{-}CH_3 + H_2O$$

The following typical esterification procedure (using BCl_3-methanol) is intended as a guideline. It may need to be altered to meet the needs of a specific application.

1. Samples can be derivatized neat or after dissolving in solvent. If appropriate, dissolve sample in a non-polar solvent (such as hexane, heptane, or toluene). If the sample is in an aqueous solvent, first evaporate to dryness then use neat or dissolved in an organic, non-polar solvent.
2. Weigh 1-25 mg of sample into a 5-10 mL micro reaction vessel.
3. Add 2 mL BCl_3-methanol, 12% w/w. A water scavenger (such as 2,2-dimethoxypropane) can be added at this point.
4. Heat at 60 °C for 5-10 minutes. Derivatization times may vary, depending on the specific compound(s) being derivatized.
5. Cool, then add 1 mL water and 1 mL hexane.
6. Shake the reaction vessel (it is critical to get the esters into the non-polar solvent).
7. After allowing the layers to settle, carefully transfer the upper (organic) layer to a clean vial. Dry the organic layer by either:
   a. *Passing through a bed of anhydrous sodium sulfate during the transfer step to the clean vial.*
   b. *Adding anhydrous sodium sulfate to the clean vial then shaking.*
8. To determine the proper derivatization time, analyze aliquots of a representative sample using different derivatization times. Plot peak area (y-axis) vs derivatization time (x-axis). The minimum time to use is when no further increase in peak area is observed with increasing derivatization time (where the curve becomes flat).
9. If it is suspected that complete derivatization is never achieved, use additional reagent or re-evaluate temperature.
10. It is important to prepare a reagent blank, along with the samples, to identify any issues that may arise.

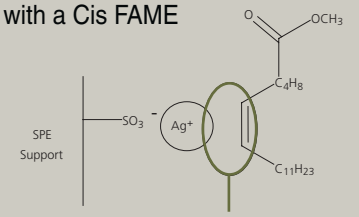
It is important to use only high quality derivatization reagents, to ensure that no artifacts are present during analysis. Additionally, only derivatization reagents with low moisture should be used, as the esterification reaction will be hindered by the presence of water. The storage conditions of derivatization reagents should be strictly adhered to, as some are susceptible to degradation during long-term storage. (5-6)

Description	Pkg. Size	Cat. No.
Derivatization Reagents		
BCl_3-Methanol, 12% w/w	20 x 1 mL	**33353**
BCl_3-Methanol, 12% w/w	20 x 2 mL	**33089-U**
BCl_3-Methanol, 12% w/w	400 mL	**33033**
BF_3-Methanol, 10% w/w	20 × 1 mL	**33356**
BF_3-Methanol, 10% w/w	19 × 2 mL	**33020-U**
BF_3-Methanol, 10% w/w	10 × 5 mL	**33040-U**
BF_3-Methanol, 10% w/w	400 mL	**33021**
BF_3-Butanol, 10% w/w	10 × 5 mL	**33126-U**
BF_3-Butanol, 10% w/w	100 mL	**33125-U**
Methanolic Base, 0.5 N	30 mL	**33352**
Methanolic Base, 0.5 N	100 mL	**33080**
Methanolic HCl, 0.5 N	20 × 1 mL	**33354**
Methanolic HCl, 0.5 N	10 × 5 mL	**33095**
Methanolic HCl, 3 N	20 × 1 mL	**33355**
Methanolic HCl, 3 N	10 × 3 mL	**33051**
Methanolic HCl, 3 N	400 mL	**33050-U**
Methanolic H_2SO_4, 10% v/v	6 × 5 mL	**506516**
Micro Reaction Vessels and Caps		
5 mL Clear, with Hole Caps	12 ea	**33299**
5 mL Clear, with Solid Caps	12 ea	**27039**
5 mL Amber, with Hole Caps	12 ea	**27478-U**
10 mL Clear, with Hole Caps	12 ea	**27479**
Water Scavenger		
2,2-Dimethoxypropane, 98%	25 mL	**D136808-25ML**
Sodium Sulfate, Anhydrous, >=99.0%		
Granular	500 g	**239313-500G**
Granular	1 Kg	**239313-1KG**
Granular	2.5 Kg	**239313-2.5KG**

FAME Fractionation Using Silver-Ion SPE Tubes

Discovery® Ag-Ion SPE tubes are based on silver-ion chromatography work first pioneered in 1966. As depicted in Figure 5, when silver ions are anchored onto SCX SPE functional group as counter-ions through electrostatic interaction, they have the ability to form polar complexes with the double bonds of unsaturated FAMEs under normal-phase conditions. More specifically, pi electrons of the FAME double bonds act as electron donors and silver-ions act as electron acceptors.

Figure 5. Schematic Representation of Ag-Ion SPE Interacting with a Cis FAME



Charge-transfer complex between Ag+ and unsaturated bond

G003513

The strength of the interactions between FAMEs and the silver counter-ions varies depending on the structure of the FAME:

- Saturated FAMEs (no double bonds) have no interactions. Therefore, they are poorly retained.
- Cis double bonds offer more steric accessibility than their trans counter part, and therefore form stronger polar complexes. As a result, cis fatty acids are more strongly retained than trans fatty acids.
- FAMEs with a greater number of double bonds have stronger interactions than those with fewer double bonds. Trienes are retained stronger than dienes, which are retained stronger than monoenes.

The differences in the strengths of these polar complexes between classes of FAMEs and the silver counter-ions can be exploited, allowing for fractionation of cis and trans isomers by adjusting the elution solvent strength. Figure 6 shows GC analyses of microwave popcorn fatty acids as FAMEs, without SPE and also with SPE fractionation. As observed, changes in the strength of the elution solvent result in 'cleaner' chromatograms of FAME classes, useful for the detailed analysis of geometric isomers.

The recovery distribution of selected C18 FAMEs in each fraction, shown in Table 1, indicates the effectiveness of Discovery Ag-Ion SPE tubes for the fractionation of cis/trans FAMEs (strength of the interaction is greater for cis FAMEs than for trans FAMEs) and also for the fractionation of FAMEs by degree of unsaturation (strength of the interaction increases with increasing number of double bonds).

Figure 6. GC Analysis of Microwave Popcorn FAMEs

sample/matrix: 1.0 g of oil scraped from a popped bag of microwave popcorn was mixed with 8 mL DI water, liquid-liquid extracted with petroleum ether, methylated with BF_3-methanol, liquid-liquid extracted with hexane, concentrated to 5 mL, then dried with sodium sulfate (complete procedure can be found in T406062 IRV)
SPE tube: Discovery Ag-Ion, 750 mg/6 mL
conditioning: 4 mL acetone followed by 4 mL hexane
sample addition: 1 mL of extract in hexane
elution: (fraction 1): 6 mL hexane:acetone (96:4)
elution: (fraction 2): 4 mL hexane:acetone (90:10)
elution: (fraction 3): 4 mL acetone
column: SP-2560, 75 m x 0.18 mm I.D., 0.14 µm (23348-U)
oven: 180 °C
inj.: 220 °C
det.: FID, 220 °C
carrier gas: hydrogen, 40 cm/sec
injection: 0.5 µL, 100:1 split
liner: 4 mm I.D., split, cup design

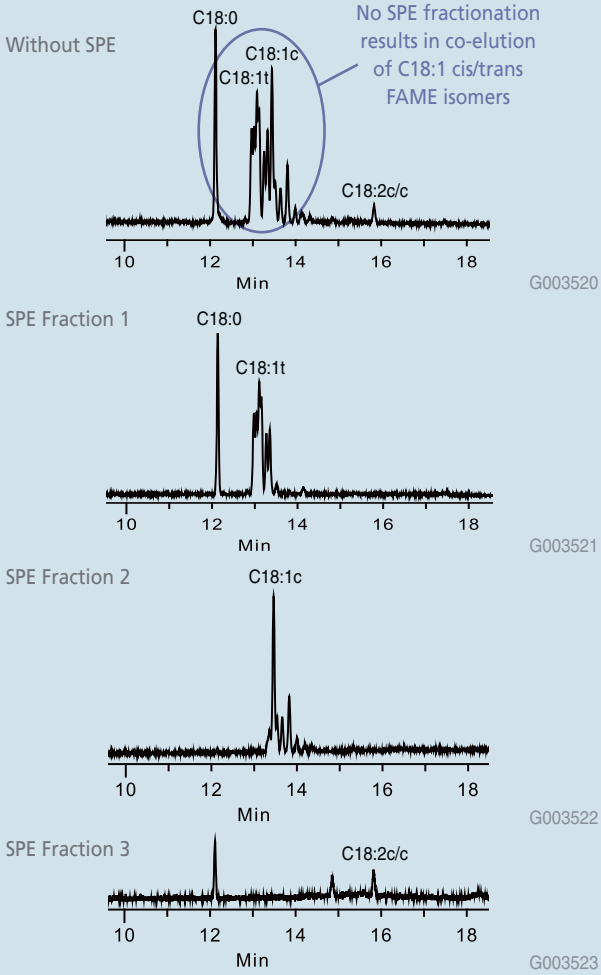


Table 1. Recovery Distribution of Selected C18 FAMEs by Fraction

Fraction	C18:0	C18:1 Trans	C18:1 Cis	C18:2 Cis/Cis
1	100%	100%	2%	----
2	----	----	98%	----
3	----	----	----	100%

Description	Pkg. Size	Cat. No.
750 mg/6mL SPE Tube	30	**54225-U**
750 mg/1mL Rezorian™ Cartridge	10	**54226-U**

FAMEs by Boiling Point Elution

The analysis of FAMEs by boiling point elution is used for pattern recognition. This technique is useful for:

- Determining the source of fatty acids when compared to patterns/profiles from known references, each with a unique fatty acid distribution. Qualitative and quantitative analysis is fundamental to food manufacturers for quality control, purity determination, and for the detection of adulterants.
- Observing subtle differences from sample to sample, which allows the effects on fatty acid metabolism, caused by either external or internal influences, to be detected. This growing area of research is commonly referred to as metabolomics, and extends to compound classes beyond fatty acids.

GC Column Choices

The separation of analytes in a boiling point elution requires the use of a non-polar GC column. The Equity-1, a rugged non-polar column, can be used for this application with great success. For application, USP code, polymer, and temperature limit information, as well as catalog numbers, please refer to page 21.

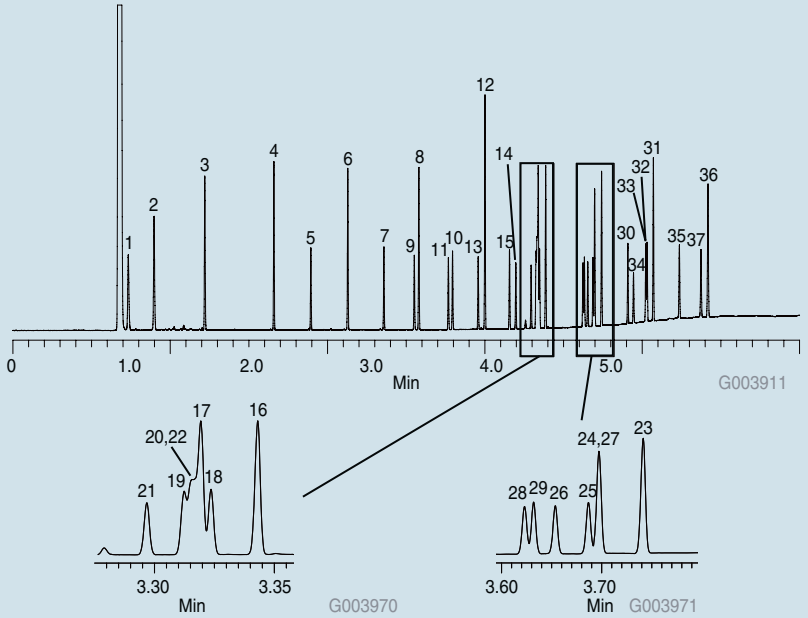
Chromatograms

The following selected chromatograms for this application are presented here to assist the chromatographer in establishing analytical conditions. For assistance, contact Supelco Technical Service at 800-359-3041 (US and Canada only), 814-359-3041, or at ***techservice@sial.com***

Figure 7. 37-Component FAME Mix on the Equity-1

column: Equity-1, 15 m x 0.10 mm I.D., 0.10 µm (28039-U)
oven: 100 °C, 50 °C/min. to 300 °C (1 min.)
inj.: 250 °C
det.: FID, 300 °C
carrier gas: hydrogen, 50 cm/sec constant
injection: 0.2 µL, 200:1 split
liner: 4 mm I.D., split, cup design
sample: Supelco 37-Component FAME Mix (47885-U), analytes at concentrations indicated in methylene chloride

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

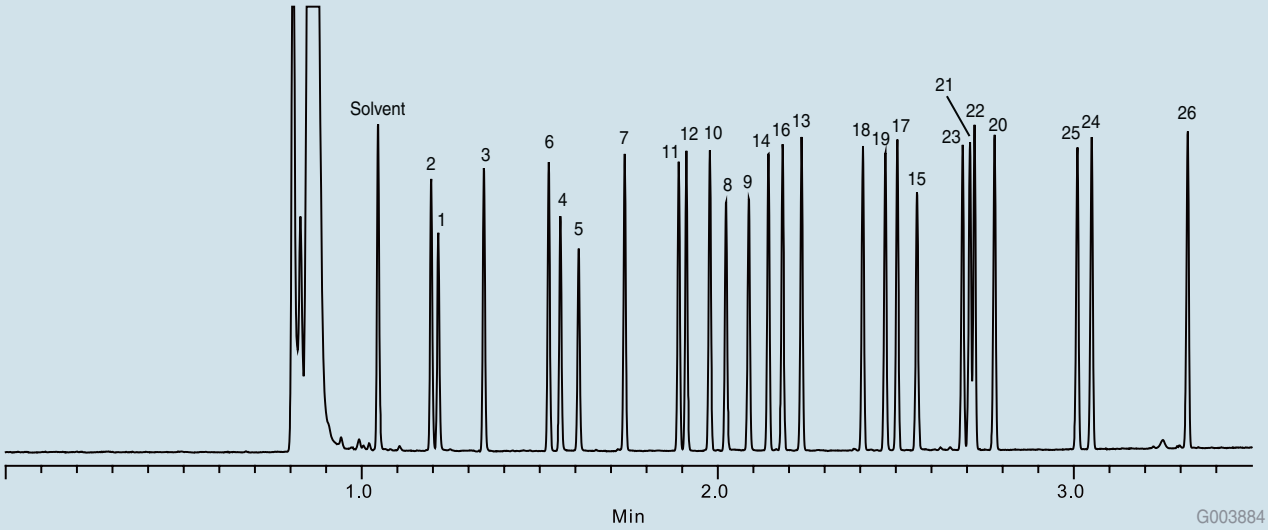


FAMEs by Boiling Point Elution

Figure 8. Bacterial Acid Methyl Esters (BAMEs) on the Equity-1

column: Equity-1, 15 m x 0.10 mm I.D., 0.10 µm (28039-U)
oven: 175 °C, 30 °C/min. to 275 °C (1 min.)
inj.: 280 °C
det.: FID, 280 °C
carrier gas: hydrogen, 45 cm/sec constant
injection: 0.5 µL, 200:1 split
liner: 4 mm I.D., split, cup design
sample: Bacterial Acid Methyl Ester (BAME) Mix (47080-U), methyl ester derivatives total concentration of 10 mg/mL in methyl caproate

1. Methyl 2-hydroxydecanoate (2-OH-C10:0)
2. Methyl undecanoate (C11:0)
3. Methyl dodecanoate (C12:0)
4. Methyl 2-hydroxydodecanoate (2-OH-C12:0)
5. Methyl 3-hydroxydodecanoate (3-OH-C12:0)
6. Methyl tridecanoate (C13:0)
7. Methyl tetradecanoate (C14:0)
8. Methyl 2-hydroxytetradecanoate (2-OH-C14:0)
9. Methyl 3-hydroxytetradecanoate (3-OH-C14:0)
10. Methyl pentadecanoate (C15:0)
11. Methyl 13-methyltetradecanoate (i-C15:0)
12. Methyl 12-methyltetradecanoate (α-C15:0)
13. Methyl hexadecanoate (C16:0)
14. Methyl 14-methylpentadecanoate (i-C16:0)
15. Methyl-2-hydroxyhexadeanoate (2-OH-C16:0)
16. Methyl cis-9-hexadecenoate ($C16:1^{9}$)
17. Methyl heptadecanoate (C17:0)
18. Methyl 15-methylhexadecanoate (i-C17:0)
19. Methyl cis-9,10-methylenehexadecanoate ($C17:0^{\Delta}$)
20. Methyl octadecanoate (C18:0)
21. Methyl cis-9-octadecenoate ($C18:1^{9}$)
22. Methyl trans-9-octadecenoate ($C18:1^{9}$) and Methyl cis-11-octadecanoate ($C18:1^{11}$)
23. Methyl cis-9,12-octadecadienoate ($C18:2^{9,12}$)
24. Methyl nonadecanoate (C19:0)
25. Methyl cis-9,10-methyleneoctadecanoate ($C19:0^{\Delta}$)
26. Methyl eicosanoate (C20:0)



Chemical Standards

To assign identification when performing the boiling point elution of fatty acid methyl esters for pattern recognition, standards of known reference must be used. To assist in confirming identification, Sigma-Aldrich offers the following chemical standards. One standard is the Supelco 37-Component FAME Mix (47885-U). This standard contains methyl esters of fatty acids ranging from C4 to C24, including key monounsaturated and polyunsaturated fatty acids, making this standard very useful to food analysts since it can be used to identify fatty acids in many different types of foods.

Characterized Reference Oils are offered that can be used as controls or check samples, providing an excellent means of standardizing applications and comparing results to others. AOCS Animal and Vegetable Reference Mixes are also available. Each quantitative mix is similar to the fatty acid distribution of certain oils, as specified in Table 2, and conforms to the requirements of AOCS Method Ce 1-62. (7)

Table 2. AOCS Animal and Vegetable Reference Mixes

Mix	Oils with Similar Fatty Acid Distribution
AOCS No. 1	Corn, cottonseed, kapok, poppyseed, rice, safflower, sesame, soybean, sunflower, and walnut
AOCS No. 2	Hempseed, linseed, perilla, and rubberseed
AOCS No. 3	Mustard seed, peanut, and rapeseed
AOCS No. 4	Neatsfoot, olive, and teaseed
AOCS No. 5	Babassu, coconut, ouri-curi, and palm kernel
AOCS No. 6	Lard, beef tallow, mutton tallow, and palm

Description	Cat. No.
Supelco 37-Component FAME Mix 10 mg/mL (total wt.) in methylene chloride, 1 mL See Figure 7 for list of analytes and concentrations	47885-U
Bacterial Acid Methyl Ester (BAME) Mix 10 mg/mL (total wt.) in methyl caproate, 1 mL qualitative standard (individual wt. % not available) See Figure 8 for a representative distribution	47080-U
Characterized Reference Oils	
Canola Oil, 1 g	46961
Coconut Oil, 1 g	46949
Corn Oil, 1 g	47112-U
Cottonseed Oil, 1 g	47113
Lard Oil, 1 g	47115-U
Linseed (Flaxseed) Oil, 1 g	47559-U
Menhaden Fish Oil, 1 g	47116
Olive Oil, 1 g	47118
Palm Oil, 1 g	46962
Peanut Oil, 1 g	47119
Safflower Oil, 1 g	47120-U
Soybean Oil, 1 g	47122
Sunflower Seed Oil, 1 g	47123
AOCS Animal and Vegetable Reference Mixes	
AOCS No.1, 100 mg	O7006-1AMP
AOCS No.2, 100 mg	O7131-1AMP
AOCS No.3, 100 mg	O7256-1AMP
Rapeseed Oil Reference Mix, 100 mg *Modern low erucic acid oil*	O7756-1AMP
AOCS No.4, 100 mg	O7381-1AMP
AOCS No.5, 100 mg	O7506-1AMP
AOCS No.6, 100 mg	O7631-1AMP

	Methyl Ester (% composition by weight)														
Description	C8:0 (caprylate)	C10:0 (caprate)	C12:0 (laurate)	C14:0 (myristate)	C16:0 (palmitate)	C16:1 (palmitoleate)	C18:0 (stearate)	C18:1 (oleate)	C18:2 (linoleate)	C18:3 (linolenate)	C20:0 (arachidate)	C20:1 (eicosenoate)	C22:0 (behenate)	C22:1 (erucate)	C24:0 (lignocerate)
AOCS No. 1					6.0		3.0	35.0	50.0	3.0	3.0				
AOCS No. 2					7.0		5.0	18.0	36.0	34.0					
AOCS No. 3				1.0	4.0		3.0	45.0	15.0	3.0	3.0		3.0	20.0	3.0
AOCS No. 4					11.0		3.0	80.0	6.0						
AOCS No. 5	7.0	5.0	48.0	15.0	7.0		3.0	12.0	3.0						
AOCS No. 6				2.0	30.0	3.0	14.0	41.0	7.0	3.0					
AOCS for Low Erucic Rapeseed Oil				1.0	4.0		3.0	60.0	12.0	5.0	3.0	1.0	3.0	5.0	3.0

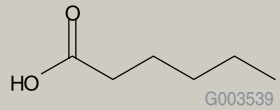
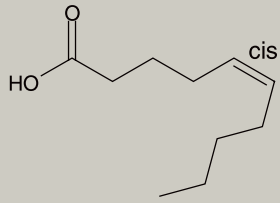
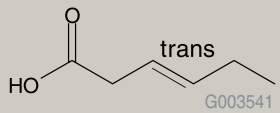
FAMEs by Degree of Unsaturation

Saturated, monounsaturated, polyunsaturated, and cis/trans configuration all refer to the structure of fatty acid moieties. Some of these structures are shown in Table 3, along with common sources and potential health effects. Because of this, it is important for food manufacturers to measure and report their levels so consumers have the chance to establish healthy dietary strategies.

Nutritionally, saturated fats are of particular concern, because an excess in the diet leads to their accumulation in the cardiovascular system, resulting in several health-related problems. Due to this, food manufacturers typically report the saturated fat vs. unsaturated fat content on the nutritional panel, allowing consumers wishing to have a healthier diet to make food choices with less saturated fat.

This section (pages 10-12) focuses on applications to determine the degree of unsaturation. Applications to determine the position of unsaturation are covered on pages 13-15. Applications to determine the cis/trans configuration of unsaturation are covered on pages 16-20.

Table 3. Types of Fatty Acids

Structure	Common Sources	Health Effects
Saturated Fatty Acids (no double bonds)  G003539	Palm kernel, Palm oil, Coconut (tropical oils), Butter, Hydrogenated Oils and Shortenings	Raise LDL cholesterol and increase risk of cardiovascular disease
Mono and Polyunsaturated Cis Fatty Acids (≥ 1 cis double bond)  G003540	Fluid/Liquid oils such as Soybean, Canola, Olive, Sunflower, and Corn	Lower LDL cholesterol, associated with reduced risk of cardiovascular disease
Mono and Polyunsaturated Trans Fatty Acids (≥ 1 trans double bond)  G003541	Partially Hydrogenated Oils, Shortenings and Margarines	Raise LDL cholesterol, like saturated fat, may also lower HDL. Associated with increased cardiovascular disease and possible type II diabetes

GC Column Choices

Determining the degree of fatty acid unsaturation of a product is difficult because foods can contain a complex mixture of saturated, monounsaturated, and polyunsaturated fatty acids with a variety of carbon chain lengths.

- Milk and butter contain saturated C4 to C20, monounsaturated C16 and C18, and polyunsaturated C18 fatty acids.
- Vegetable oils contain saturated C6 to C24, monounsaturated C16, and monounsaturated cis C18, C20, and C22 fatty acids.
- Margarines contain the same fatty acids as vegetable oils plus monounsaturated trans C18, C20, and C22, and polyunsaturated C18 fatty acids.
- Fish and meat typically contain saturated and monounsaturated C12 to C24+ fatty acids, plus polyunsaturated omega 3 C18, C20, and C22, and polyunsaturated omega 6 C18 and C20 fatty acids.
- Fish tends to be richer in the polyunsaturated omega 3 fatty acids, whereas meats are richer in the polyunsaturated omega 6 fatty acids.

To confirm identification, very efficient capillary GC columns with the ability to resolve a large number of peaks are required.

- Omegawax columns provide highly reproducible analyses, being specially tested for reproducibility of FAME equivalent chain length (ECL) values and resolution of key components.
- The SLB-IL100 column exhibits one of the highest polarities of any GC phase, providing an alternative selectivity for FAME applications typically performed on Omegawax columns.

For application, USP code, polymer, and temperature limit information, as well as catalog numbers, please refer to page 22.

Chromatograms

The following selected chromatograms for this application are presented here to assist the chromatographer in establishing analytical conditions. For assistance, contact Supelco Technical Service at 800-359-3041 (US and Canada only), 814-359-3041, or at ***techservice@sial.com***

Figure 9. 37-Component FAME Mix on the Omegawax 100

column: Omegawax 100, 15 m x 0.10 mm I.D., 0.10 µm (23399-U)
oven: 140 °C, 40 °C/min. to 280 °C (2 min.)
inj.: 250 °C
det.: FID, 260 °C
carrier gas: hydrogen, 50 cm/sec constant
injection: 0.2 µL, 200:1 split
liner: 4 mm I.D., split, cup design
sample: Supelco 37-Component FAME Mix (47885-U), analytes at concentrations indicated in methylene chloride

See Figure 7 for list of analytes and concentrations

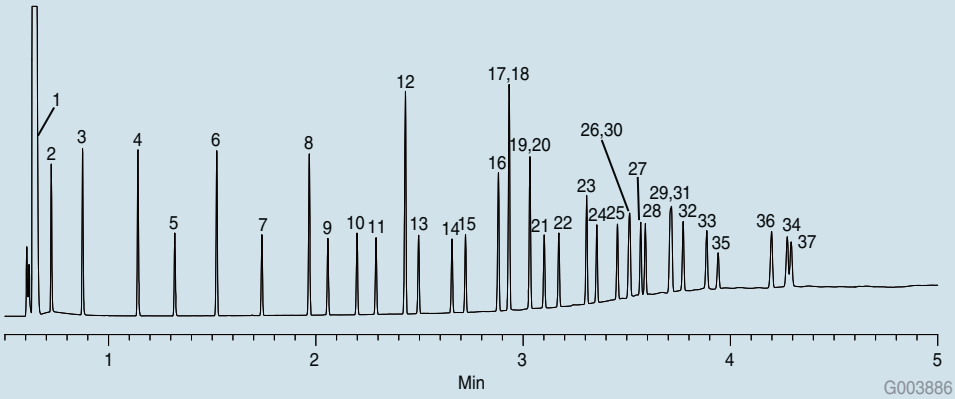


Figure 10. 37-Component FAME Mix on the Omegawax 250

column: Omegawax 250, 30 m x 0.25 mm I.D., 0.25 µm (24136)
oven: 50 °C (2 min.), 4 °C/min. to 220 °C (15 min.)
inj.: 250 °C
det.: FID, 260 °C
carrier gas: helium, 30 cm/sec @ 205 °C
injection: 1 µL, 100:1 split
sample: Supelco 37-Component FAME Mix (47885-U), analytes at concentrations indicated in methylene chloride

See Figure 7 for list of analytes and concentrations

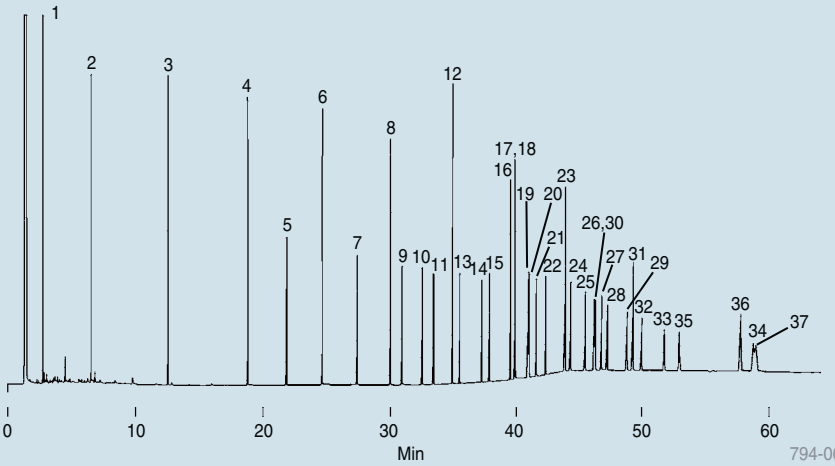
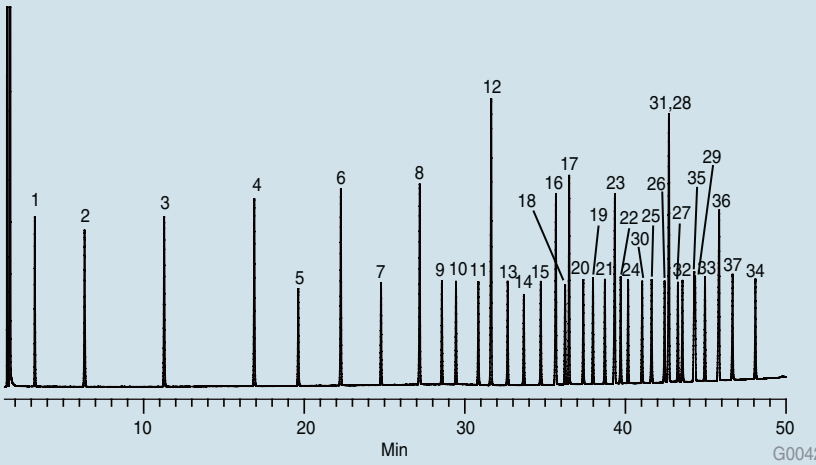


Figure 11. 37-Component FAME Mix on the 30 m SLB-IL100

Chromatogram courtesy of Prof. Luigi Mondello (Univ. of Messina, Italy)

column: SLB-IL100, 30 m x 0.25 mm I.D., 0.20 µm (28884-U)
oven: 50 °C, 3.0 °C/min. to 240 °C
inj.: 240 °C
det.: FID, 240 °C
carrier gas: helium, 40 cm/sec constant
injection: 1 µL, 50:1 split
sample: Supelco 37-Component FAME Mix (47885-U), analytes at concentrations indicated in methylene chloride

See Figure 7 for list of analytes and concentrations

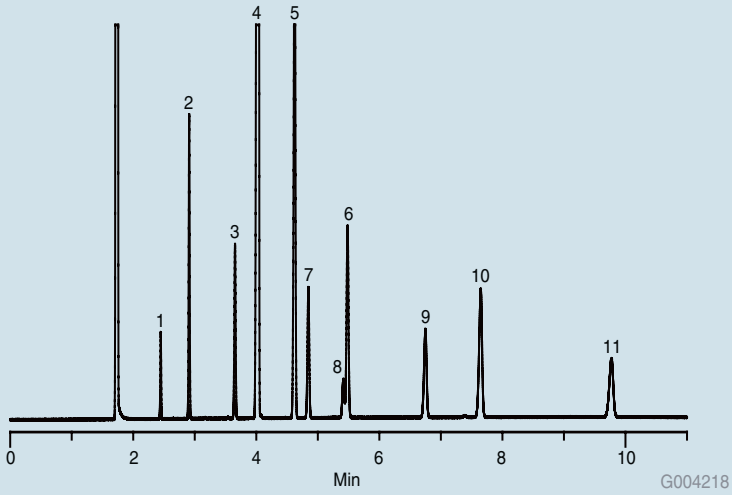


FAMEs by Degree of Unsaturation

Figure 12. Rapeseed Oil FAMEs on the SLB-IL100

column: SLB-IL100, 30 m x 0.25 mm I.D., 0.20 µm (28884-U)
oven: 180 °C
inj.: 250 °C
det.: FID, 250 °C
carrier gas: helium, 30 cm/sec @ 180 °C
injection: 1 µL, 100:1 split
liner: 4 mm I.D., split, cup
sample: Rapeseed oil FAME mix, 5 mg/mL total FAMEs in methylene chloride

1. C14:0
2. C16:0
3. C18:0
4. C18:1n9c
5. C18:2
6. C18:3
7. C20:0
8. C20:1
9. C22:0
10. C22:1
11. C24:0



Chemical Standards

To assist in assigning identifications based on degree of unsaturation, Sigma-Aldrich offers the following standards. One standard is the Supelco 37-Component FAME Mix (47885-U). This standard contains methyl esters of fatty acids ranging from C4 to C24, including key monounsaturated and polyunsaturated fatty acids, making this standard very useful to food analysts since it can be used to identify fatty acids in many different types of foods.

Several convenient kits of either derivatized FAMEs or underivatized fatty acids are also offered, so analysts can formulate their own mixes. These kits contain each individual analyte in a separate vial, with all vials contained in a sturdy storage box.

Description			Cat. No.
Supelco 37-Component FAME Mix 10 mg/mL (total wt.) in methylene chloride, 1 mL *See Figure 7 for list of analytes and concentrations*			47885-U
C6-C24, Even Carbon Number, Saturated FAMEs Kit 10 individual vials, one analyte per vial *Caproic Acid Methyl Ester (C6:0), 1 g* *Caprylic Acid Methyl Ester (C8:0), 1 g* *Capric Acid Methyl Ester (C10:0), 1 g* *Lauric Acid Methyl Ester (C12:0), 1 g*	*Myristic Acid Methyl Ester (C14:0), 1 g* *Palmitic Acid Methyl Ester (C16:0), 1 g* *Stearic Acid Methyl Ester (C18:0), 1 g*	*Arachidic Acid Methyl Ester (C20:0), 1 g* *Behenic Acid Methyl Ester (C22:0), 1 g* *Lignoceric Acid Methyl Ester (C24:0), 1 g*	ME10-1KT
C6-C24, Even Carbon Number, Saturated Fatty Acid Kit 10 individual vials, one analyte per vial *Caproic Acid (C6:0), 10 mL* *Caprylic Acid (C8:0), 10 mL* *Capric Acid (C10:0), 10 g* *Lauric Acid (C12:0), 10 g*	*Myristic Acid (C14:0), 10 g* *Palmitic Acid (C16:0), 10 g* *Stearic Acid (C18:0), 10 g*	*Arachidic Acid (C20:0), 10 g* *Behenic Acid (C22:0), 10 g* *Lignoceric Acid (C24:0), 10 g*	EC10-1KT
C6-C24 Saturated FAMEs Kit 19 individual vials, one analyte per vial *Caproic Acid Methyl Ester (C6:0), 1 g* *Heptanoic Acid Methyl Ester (C7:0), 1 g* *Caprylic Acid Methyl Ester (C8:0), 1 g* *Nonanoic Acid Methyl Ester (C9:0), 1 g* *Capric Acid Methyl Ester (C10:0), 1 g* *Undecanoic Acid Methyl Ester (C11:0), 1 g* *Lauric Acid Methyl Ester (C12:0), 1 g*	*Tridecanoic Acid Methyl Ester (C13:0), 1 g* *Myristic Acid Methyl Ester (C14:0), 1 g* *Pentadecanoic Acid Methyl Ester (C15:0), 1 g* *Palmitic Acid Methyl Ester (C16:0), 1 g* *Heptadecanoic Acid Methyl Ester (C17:0), 1 g* *Stearic Acid Methyl Ester (C18:0), 1 g*	*Nonadecanoic Acid Methyl Ester (C19:0), 1 g* *Arachidic Acid Methyl Ester (C20:0), 1 g* *Heneicosanoic Acid Methyl Ester (C21:0), 1 g* *Behenic Acid Methyl Ester (C22:0), 1 g* *Tricosanoic Acid Methyl Ester (C23:0), 1 g* *Lignoceric Acid Methyl Ester (C24:0), 1 g*	ME19-1KT
C24-C31 Saturated FAMEs Kit 7 individual vials, one analyte per vial *Lignoceric Acid Methyl Ester (C24:0), 1 g* *Pentacosanoic Acid Methyl Ester (C25:0), 1 g* *Hexacosanoic Acid Methyl Ester (C26:0), 100 mg* *Heptacosanoic Acid Methyl Ester (C27:0), 100 mg*	*Octocosanoic Acid Methyl Ester (C28:0), 100 mg* *Triacontanoic Acid Methyl Ester (C30:0), 100 mg* *Hentriacontanoic Acid Methyl Ester (C31:0), 100 mg*		ME7-1KT

Omega 3 and Omega 6 Fatty Acids as FAMEs

Essential fats are nutrients that must be obtained from the diet because humans lack the anabolic processes for their synthesis. Essential fats serve multiple purposes in the body including:

- Production of eicosanoids, which affect inflammation and cellular function.
- Production of lipoxins and resolvins, which affect inflammation.
- Production of endogenous cannabinoids, which affect mood and behavior.
- Influencing cell signaling.
- Regulation of blood pressure, blood clotting, lipid levels, immune response, and gene expression.

There are two closely related groups of essential fats, the omega 3 and omega 6 fatty acids. Both are unsaturated fatty acids, with the initial double bond located directly after the third (omega 3) or the sixth (omega 6) carbon atom as measured from the methyl end. Omega 3 fatty acids are found in fish oils and some nut oils. Seed oils are the primary dietary source of omega 6 fatty acids.

Before the advent of agriculture, human diets were thought to have consisted of an equal amount of omega 3 and omega 6 fatty acids. In contrast, the current western diet has a 1:7 ratio of omega 3 to omega 6 fatty acids. Low levels of omega 3 fatty acids, or an altered ratio of omega 3 to omega 6 fatty acids, may play a key role in a number of human diseases:

- Increased consumption of omega 3 fatty acids has been linked with reducing coronary heart disease.
- An excess of omega 6 fatty acids can interfere with the health benefits of omega 3 fatty acids, and has also been linked with several detrimental health conditions.

As a result of consumers' desire to have 'healthier fat' in the diet, the analysis of the omega 3 and omega 6 fatty acid content of food products has become a very active area of research for many food companies.

GC Column Choices

The omega 3 and omega 6 FAMEs may have very similar physical (such as boiling point) and chemical (such as chain length) properties as other FAMEs that may be present in the sample. Therefore, specialized GC columns with the ability to resolve these specific FAMEs are required for proper identification.

- Omegawax columns provide highly reproducible analyses, being specially tested for reproducibility of FAME equivalent chain length (ECL) values and resolution of key components, specifically the omega 3 and omega 6 FAMEs. This column is specified in AOAC Method 991.39 and AOCS Method Ce 1b-89. (8-9)
- The SLB-IL100 column exhibits one of the highest polarities of any GC phase, providing an alternative selectivity for FAME applications typically performed on Omegawax columns.

For application, USP code, polymer, and temperature limit information, as well as catalog numbers, please refer to page 22.

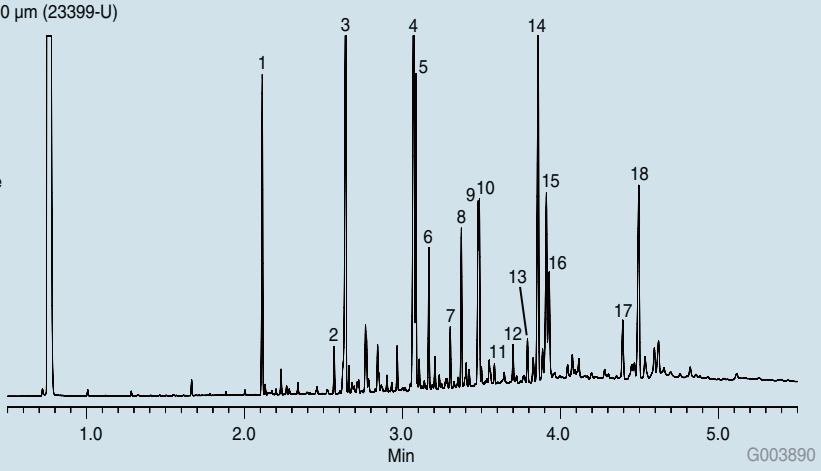
Chromatograms

The following selected chromatograms for this application are presented here to assist the chromatographer in establishing analytical conditions. For assistance, contact Supelco Technical Service at 800-359-3041 (US and Canada only), 814-359-3041, or at ***techservice@sial.com***

Figure 13. Marine Source FAMEs on the Omegawax 100

column: Omegawax 100, 15 m x 0.10 mm I.D., 0.10 µm (23399-U)
oven: 140 °C, 40 °C/min. to 280 °C (2 min.)
inj.: 250 °C
det.: FID, 280 °C
carrier gas: hydrogen, 50 cm/sec constant
injection: 0.2 µL, 200:1 split
liner: 4 mm I.D., split, cup design
sample: PUFA No. I - Marine Source (47033), diluted to 50 mg/mL in methylene chloride

1. C14:0
2. C16:0
3. C16:1n7
4. C18:1n9
5. C18:1n7
6. C18:2n6
7. C18:3n3
8. C18:4n3
9. C20:1n11
10. C20:1n9
11. C20:1n7
12. C20:4n6
13. C20:4n3
14. C20:5n3
15. C22:1n11
16. C22:1n9
17. C22:5n3
18. C22:6n3



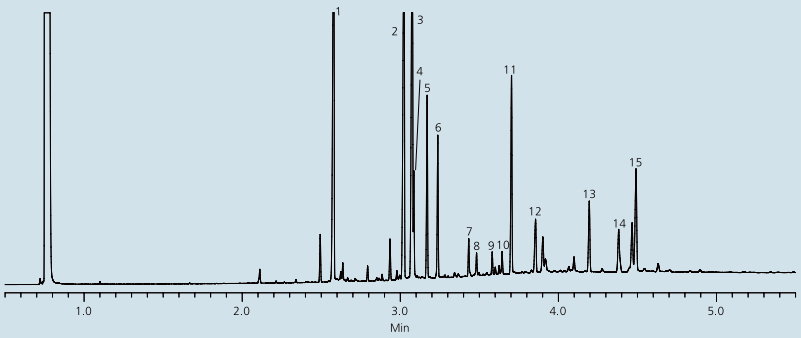
Omega 3 and Omega 6 Fatty Acids as FAMEs

Chromatograms

Figure 14. Animal Source FAMEs on the Omegawax 100

column: Omegawax 100, 15 m x 0.10 mm I.D., 0.10 µm (23399-U)
oven: 140 °C, 40 °C/min. to 280 °C (2 min.)
inj.: 250 °C
det.: FID, 280 °C
carrier gas: hydrogen, 50 cm/sec constant
injection: 0.2 µL, 200:1 split
liner: 4 mm I.D., split, cup design
sample: PUFA No. II – Animal Source (47015-U), diluted to 50 mg/mL in methylene chloride

1. C16:0
2. C18:0
3. C18:1n9
4. C18:1n7
5. C18:2n6
6. C18:3n6
7. C20:0
8. C20:1n9
9. C20:2n9
10. C20:3n6
11. C20:4n6
12. C20:5n3
13. C22:5n6
14. C22:5n3
15. C22:6n3

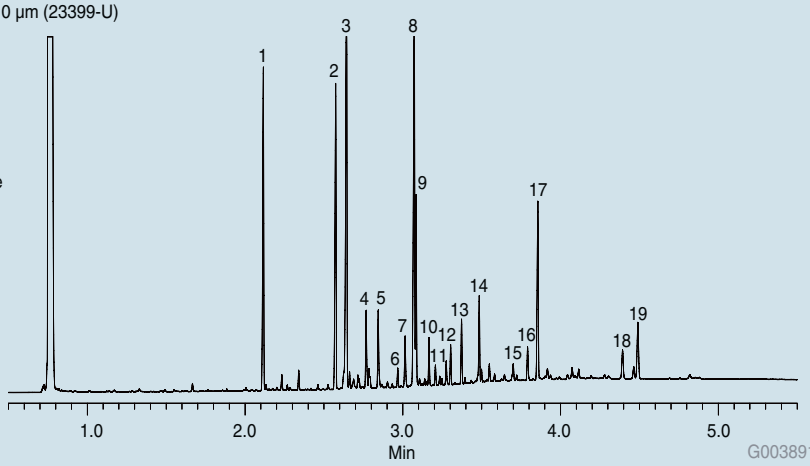


G003892

Figure 15. Menhaden Oil FAMEs on the Omegawax 100

column: Omegawax 100, 15 m x 0.10 mm I.D., 0.10 µm (23399-U)
oven: 140 °C, 40 °C/min. to 280 °C (2 min.)
inj.: 250 °C
det.: FID, 280 °C
carrier gas: hydrogen, 50 cm/sec constant
injection: 0.2 µL, 200:1 split
liner: 4 mm I.D., split, cup design
sample: PUFA No. III – Menhaden Oil (47085-U), diluted to 50 mg/mL in methylene chloride

1. C14:0
2. C16:0
3. C16:1n7
4. C16:2n4
5. C16:3n4
6. C16:4n1
7. C18:0
8. C18:1n9
9. C18:1n7
10. C18:2n6
11. C18:3n4
12. C18:3n3
13. C18:4n3
14. C20:1n9
15. C20:4n6
16. C20:4n3
17. C20:5n3
18. C22:5n3
19. C22:6n3



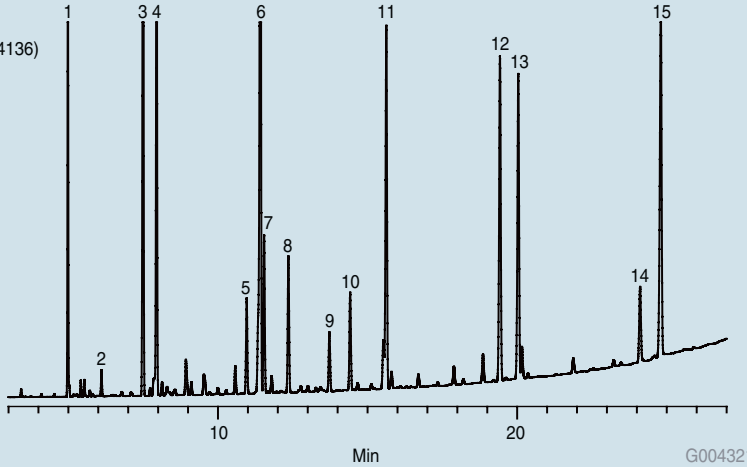
G003891

Figure 16. Cod Liver Oil FAMEs on the Omegawax 250

Chromatogram courtesy of Prof. Luigi Mondello (Univ. of Messina, Italy)

column: Omegawax 250, 30 m x 0.25 mm I.D., 0.25 µm (24136)
oven: 180 °C, 3.0 °C/min. to 270 °C
inj.: 250 °C
det.: FID, 270 °C
carrier gas: hydrogen, 35 cm/sec constant
injection: 1 µL, 50:1 split
sample: cod liver oil FAMEs

1. C14:0
2. C15:0
3. C16:0
4. C16:1n7
5. C18:0
6. C18:1n9
7. C18:1n7
8. C18:2n6
9. C18:3n3
10. C18:4n3
11. C20:1n9
12. C20:5n3
13. C22:1n9
14. C22:5n3
15. C22:6n3



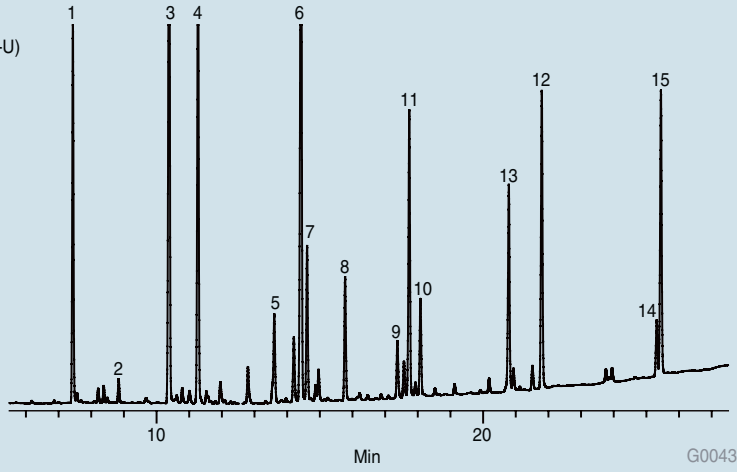
G004321

Figure 17. Cod Liver Oil FAMEs on the SLB-IL100

Chromatogram courtesy of Prof. Luigi Mondello (Univ. of Messina, Italy)

column: SLB-IL100, 30 m x 0.25 mm I.D., 0.20 µm (28884-U)
oven: 120 °C, 3.0 °C/min. to 240 °C
inj.: 240 °C
det.: FID, 240 °C
carrier gas: hydrogen, 35 cm/sec constant
injection: 1 µL, 50:1 split
sample: cod liver oil FAMEs

Same Peak IDs as Figure 16



Chemical Standards

To assist in confirming omega 3 and omega 6 identifications, Sigma-Aldrich offers the following standards. One standard is the Supelco 37-Component FAME Mix (47885-U). This standard contains methyl esters of fatty acids ranging from C4 to C24, including key monounsaturated and polyunsaturated fatty acids, making this standard very useful to food analysts since it can be used to identify fatty acids in many different types of foods.

The PUFA (polyunsaturated fatty acid) methyl ester mixes are complex qualitative standard mixtures, which can be used to verify the presence of omega 3 and omega 6 FAMEs. Because they are extracted from natural materials, relative peak sizes and compositions may vary from lot to lot.

Many omega 3 and omega 6 fatty acids and FAMEs are also available as individual compounds or standards. Each product comes with a Certificate of Analysis that includes a purity determination. Standards are prepared gravimetrically using NIST traceable weights. The availability of small package sizes eliminates the need to buy bulk material as standards.

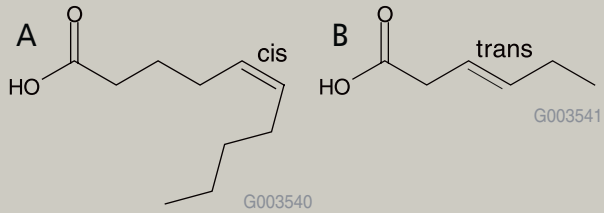
Description	Cat. No.
Supelco 37-Component FAME Mix 10 mg/mL (total wt.) in methylene chloride, 1 mL *See Figure 7 for list of analytes and concentrations*	47885-U
PUFA No. I (Marine Source) 100 mg (total wt.) qualitative standard (individual wt. % not available) *See Figure 13 for a representative distribution*	47033
PUFA No. II (Animal Source) 100 mg (total wt.) qualitative standard (individual wt. % not available) *See Figure 14 for a representative distribution*	47015-U
PUFA No. III (from Menhaden Oil) 100 mg (total wt.) qualitative standard (individual wt. % not available) *See Figure 15 for a representative distribution*	47085-U
Individual Essential Fatty Acids and FAMEs	
Linoleic Acid (C18:2n6) , 5 mL or 25 mL	62230
α-Linolenic Acid (C18:3n3), 1 mL or 5 mL	62160
γ-Linolenic Acid (C18:3n6), 100 mg or 500 mg	62174
Methyl Stearidonate Solution (C18:4n3), 100 mg/mL in ethanol	56463
cis-11,14-Eicosadienoic Acid (C20:2n6), 25 mg or 100 mg	E3127
cis-5,8,11,14-Eicosatetraenoate Acid Methyl Ester (C20:4n6), 1 mL	47572-U
Arachidonic acid, (C20:4n6), 10 mg, 50 mg, 100 mg, 500 mg, 1g	A9673
cis-5,8,11,14,17-Eicosapentaenoic Acid Methyl Ester (C20:5n3), 100 mg	17266
cis-7,10,13,16-Docosatetraenoic Acid (C22:4n6), 10 mg	49557
cis-7,10,13,16,19-Docosapentaenoic Acid Methyl Ester (C22:5n3), 50 mg	17269
cis-4,7,10,13,16-Docosapentaenoic Acid (C22:5n6), 10 mg	18566

Cis/Trans Fatty Acid Isomers as FAMEs

Fatty acids in the cis configuration (Figure 18A) are the dominant form in nature. Correspondingly, enzymes have evolved to efficiently digest and metabolize them with a high degree of specificity. Conversely, trans fatty acids (Figure 18B) are relatively rare in nature. However, because they can increase the shelf life and flavor stability of foods containing them, they have become predominant synthetic additives to processed foods, especially fried foods and baked goods.

Unfortunately, trans fatty acids, formed by partial hydrogenation of vegetable oil, interfere with natural metabolic process, resulting in an imbalance of the LDL:HDL ratio, and also increasing lipoprotein(a) levels. Studies have linked their nutritional contribution to be similar to that of saturated fatty acids, possibly playing a role in the heightened risk of coronary artery disease.

Figure 18. Structures of Cis and Trans Fatty Acids



Because trans fatty acids have adverse health consequences and no known nutritional benefits over other fats, consumer groups have pressured manufacturers and restaurants for their elimination. Many regulatory agencies worldwide now require content labeling to inform buyers of 'trans fat' levels of foods and some dietary supplements.

GC Column Choices

Because the differences between cis isomer FAMEs and trans isomer FAMEs of the same carbon length and degree of unsaturation are very small, very efficient capillary GC columns with highly polar phases are required.

- The high polarity of the SP-2380 column allows the separation of geometric (cis/trans) isomers as a group. The phase is stabilized, providing a maximum temperature slightly higher than the popular SP-2560 column.
- The very polar SP-2560 column was specifically designed for the separation of geometric-positional (cis/trans) isomers of FAMEs, and is extremely effective for special FAME applications including the separation of FAMEs in hydrogenated vegetable oil samples. This column is specified in AOAC Method 996.06 and AOCS Method Ce 1h-05. (10-11)
- The SLB-IL100 column exhibits one of the highest polarities of any GC phase, providing an alternative selectivity for FAME applications typically performed on SP-2380 and SP-2560 columns.

For application, USP code, polymer, and temperature limit information, as well as catalog numbers, please refer to page 22.

Chromatograms

The following selected chromatograms for this application are presented here to assist the chromatographer in establishing analytical conditions. For assistance, contact Supelco Technical Service at 800-359-3041 (US and Canada only), 814-359-3041, or at ***techservice@sial.com***

Figure 19. 37-Component FAME Mix on the 30 m SP-2380

column: SP-2380, 30 m x 0.25 mm I.D., 0.20 µm (24110)
oven: 50 °C (2 min.), 4 °C/min. to 250 °C (15 min.)
inj.: 250 °C
det.: FID, 260 °C
carrier gas: helium, 20 cm/sec @ 150 °C
injection: 1 µL, 100:1 split
sample: Supelco 37-Component FAME Mix (47885-U), analytes at concentrations indicated in methylene chloride

See Figure 7 for list of analytes and concentrations

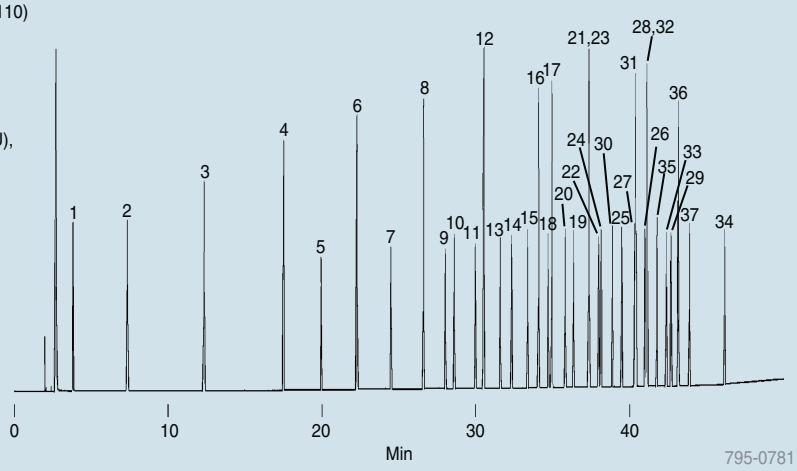


Figure 20. 37-Component FAME Mix on the 75 m SP-2560

column: SP-2560, 75 m x 0.18 mm I.D., 0.14 µm (23348-U)
oven: 140 °C (5 min.), 4 °C/min. to 240 °C (2 min.)
inj.: 250 °C
det.: FID, 250 °C
carrier gas: hydrogen, 40 cm/sec @ 175 °C
injection: 1 µL, 100:1 split
liner: 4 mm I.D, split, cup design
sample: Supelco 37-Component FAME Mix (47885-U), analytes at concentrations indicated in methylene chloride

See Figure 7 for list of analytes and concentrations

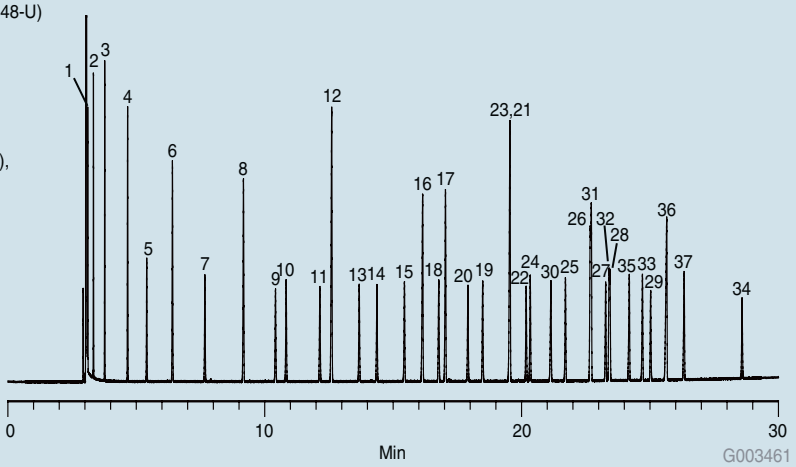


Figure 21. 37-Component FAME Mix on the 100 m SP-2560

column: SP-2560, 100 m x 0.25 mm I.D., 0.20 µm (24056)
oven: 140 °C (5 min.), 4 °C/min. to 240 °C (15 min.)
inj.: 250 °C
det.: FID, 260 °C
carrier gas: helium, 20 cm/sec @ 175 °C
injection: 1 µL, 100:1 split
sample: Supelco 37-Component FAME Mix (47885-U), analytes at concentrations indicated in methylene chloride

See Figure 7 for list of analytes and concentrations

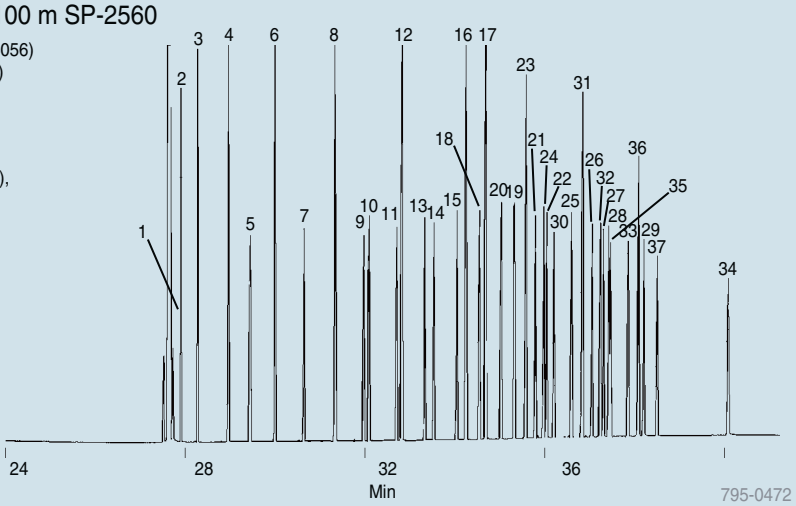
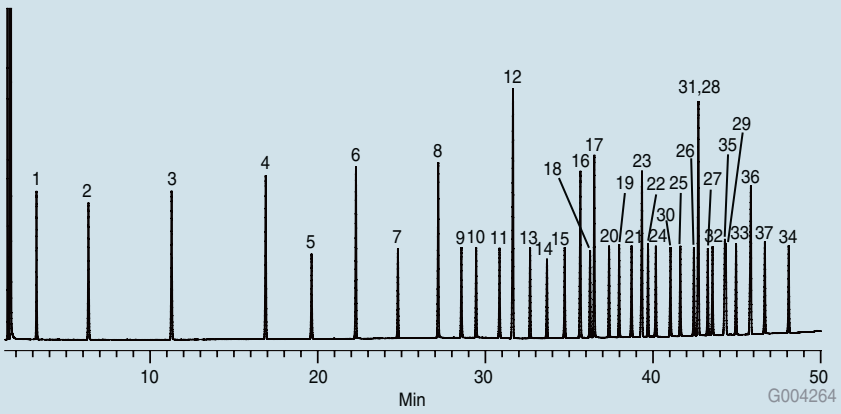


Figure 22. 37-Component FAME Mix on the 30 m SLB-IL100

Chromatogram courtesy of Prof. Luigi Mondello (Univ. of Messina, Italy)

column: SLB-IL100, 30 m x 0.25 mm I.D., 0.20 µm (28884-U)
oven: 50 °C, 3.0 °C/min. to 240 °C
inj.: 240 °C
det.: FID, 240 °C
carrier gas: helium, 40 cm/sec constant
injection: 1 µL, 50:1 split
sample: Supelco 37-Component FAME Mix (47885-U), analytes at concentrations indicated in methylene chloride

See Figure 7 for list of analytes and concentrations

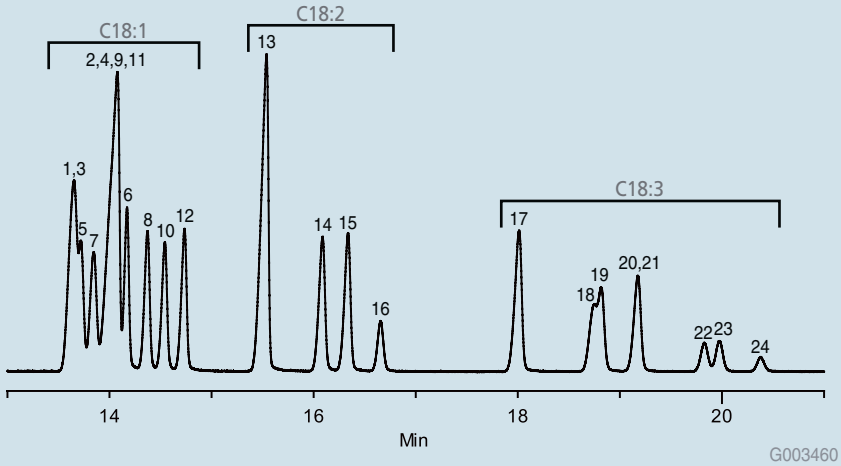


Cis/Trans Fatty Acid Isomers as FAMEs

Figure 23. Detailed Analysis of cis/trans C18 FAME Isomers on the 75 m SP-2560

column: SP-2560, 75 m x 0.18 mm I.D., 0.14 µm (23348-U)
oven: 180 °C
inj.: 220 °C
det.: FID, 220 °C
carrier gas: hydrogen, 25 cm/sec @ 180 °C
injection: 0.5 µL, 100:1 split
liner: 4 mm I.D., split, cup design
sample: Mixture of C18:1, C18:2, and C18:3 FAMEs in methylene chloride

1. C18:1 Δ 6t
2. C18:1 Δ 6c
3. C18:1 Δ 7t
4. C18:1 Δ 7c
5. C18:1 Δ 9t
6. C18:1 Δ 9c
7. C18:1 Δ 11t
8. C18:1 Δ 11c
9. C18:1 Δ 12t
10. C18:1 Δ 12c
11. C18:1 Δ 13t
12. C18:1 Δ 13c
13. C18:2 Δ 9t,12t
14. C18:2 Δ 9c,12t
15. C18:2 Δ 9t,12c
16. C18:2 Δ 9c,12c
17. C18:3 Δ 9t,12t,15t
18. C18:3 Δ 9t,12t,15c
19. C18:3 Δ 9t,12c,15t
20. C18:3 Δ 9c,12c,15t
21. C18:3 Δ 9c,12t,15t
22. C18:3 Δ 9c,12t,15c
23. C18:3 Δ 9t,12c,15c
24. C18:3 Δ 9c,12c,15c

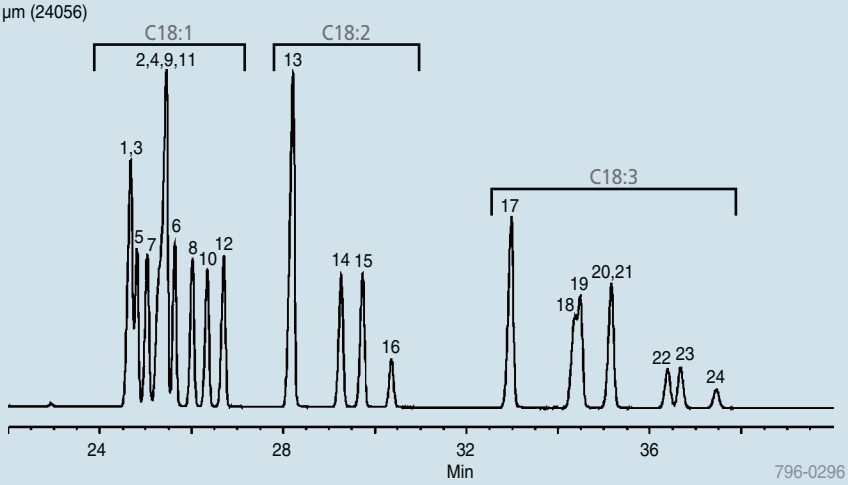


G003460

Figure 24. Detailed Analysis of cis/trans C18 FAME Isomers on the 100 m SP-2560

column: SP-2560, 100 m x 0.25 mm I.D., 0.20 µm (24056)
oven: 175 °C
inj.: 210 °C
det.: FID, 250 °C
carrier gas: helium, 20 cm/sec @ 175 °C
injection: 1.0 µL, 100:1 split
sample: Mixture of C18:1, C18:2, and C18:3 FAMEs in methylene chloride

Same Peak IDs as Figure 23

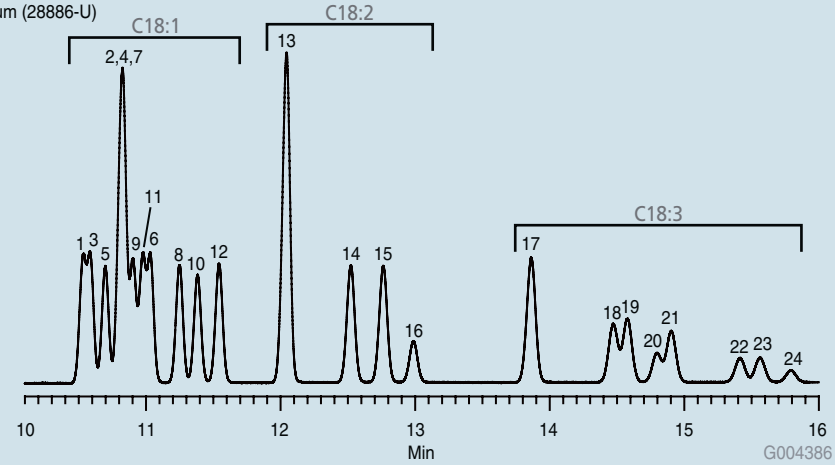


796-0296

Figure 25. Detailed Analysis of cis/trans C18 FAME Isomers on the 60 m SLB-IL100

column: SLB-IL100, 60 m x 0.25 mm I.D., 0.20 µm (28886-U)
oven: 170 °C
inj.: 250 °C
det.: FID, 250 °C
carrier gas: helium, 30 cm/sec
injection: 1 µL, 50:1 split
liner: 4 mm I.D., split, cup design
sample: Mixture of C18:1, C18:2, and C18:3 FAMEs in methylene chloride

Same Peak IDs as Figure 23



G004386

Chemical Standards

To assist in confirming cis/trans identifications, Sigma-Aldrich offers the following standards. One standard is the Supelco 37-Component FAME Mix (47885-U). This standard contains methyl esters of fatty acids ranging from C4 to C24, including key monounsaturated and polyunsaturated fatty acids, making this standard very useful to food analysts since it can be used to identify fatty acids in many different types of foods.

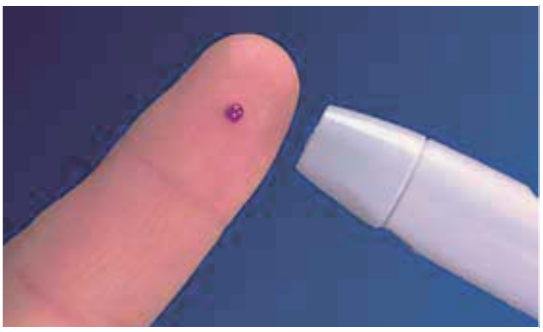
Description	Cat. No.
trans-9-Tetradecenoic Acid Methyl Ester (C14:1n9t), 100 mg	70055
trans-9-Hexadecenoic Acid Methyl Ester (C16:1n9t), 100 mg	76117
cis-6-Octadecenoic Acid Methyl Ester (C18:1n6c), 10 mg/mL in heptane, 1 mL	47198
trans-6-Octadecenoic Acid Methyl Ester (C18:1n6t), 10 mg/mL in heptane, 1 mL	47199
cis-9-Octadecenoic Acid Methyl Ester (C18:1n9c), 10 mg/mL in heptane, 1 mL	46902-U
trans-9-Octadecenoic Acid Methyl Ester (C18:1n9t), 10 mg/mL in heptane, 1 mL	46903
cis-11-Octadecenoic Acid Methyl Ester (C18:1n11c), 10 mg/mL in heptane, 1 mL	46904
trans-11-Octadecenoic Acid Methyl Ester (C18:1n11t), 10 mg/mL in heptane, 1 mL	46905-U
Methyl cis-12-Octadecenoate, (C18:1n12c), 50 mg	02817
Linoleic Acid Methyl Ester (C18:2) Isomer Mix 10 mg/mL (total wt.) in methylene chloride, 1 mL *cis-9,cis-12-Octadecadienoic Acid Methyl Ester (C18:2Δ9c,12c), ~10% w/w* *cis-9,trans-12-Octadecadienoic Acid Methyl Ester (C18:2Δ9c,12t), ~20% w/w* *trans-9,cis-12-Octadecadienoic Acid Methyl Ester (C18:2Δ9t,12c), ~20% w/w* *trans-9,trans-12-Octadecadienoic Acid Methyl Ester (C18:2Δ9t,12t), ~50% w/w*	47791
Linolenic Acid Methyl Ester (C18:3) Isomer Mix 10 mg/mL (total wt.) in methylene chloride, 1 mL *cis-9,cis-12,cis-15-Octadecatrienoic Acid Methyl Ester (C18:3Δ9c,12c,15c), ~3% w/w* *cis-9,cis-12,trans-15-Octadecatrienoic Acid Methyl Ester (C18:3Δ9c,12c,15t), ~7% w/w* *cis-9,trans-12,cis-15-Octadecatrienoic Acid Methyl Ester (C18:3Δ9c,12t,15c), ~7% w/w* *cis-9,trans-12,trans-15-Octadecatrienoic Acid Methyl Ester (C18:3Δ9c,12t,15t), ~15% w/w* *trans-9,cis-12,cis-15-Octadecatrienoic Acid Methyl Ester (C18:3Δ9t,12c,15c), ~7% w/w* *trans-9,cis-12,trans-15-Octadecatrienoic Acid Methyl Ester (C18:3Δ9t,12c,15t), ~15% w/w* *trans-9,trans-12,cis-15-Octadecatrienoic Acid Methyl Ester (C18:3Δ9t,12t,15c), ~15% w/w* *trans-9,trans-12,trans-15-Octadecatrienoic Acid Methyl Ester (C18:3Δ9t,12t,15t), ~30% w/w*	47792
Supelco 37-Component FAME Mix 10 mg/mL (total wt.) in methylene chloride, 1 mL *See Figure 7 for list of analytes and concentrations*	47885-U
C4-C24 FAME Mix Neat mixture of 37 analytes, 100 mg total wt. *Butyric Acid Methyl Ester (C4:0) at 4 wt %* *Caproic Acid Methyl Ester (C6:0) at 4 wt %* *Caprylic Acid Methyl Ester (C8:0) at 4 wt %* *Capric Acid Methyl Ester (C10:0) at 4 wt %* *Undecanoic Acid Methyl Ester (C11:0) at 2 wt %* *Lauric Acid Methyl Ester (C12:0) at 4 wt %* *Tridecanoic Acid Methyl Ester (C13:0) at 2 wt %* *Myristic Acid Methyl Ester (C14:0) at 4 wt %* *Myristoleic Acid Methyl Ester (C14:1) at 2 wt %* *Pentadecanoic Acid Methyl Ester (C15:0) at 2 wt %* *cis-10-Pentadecenoic Acid Methyl Ester (C15:1) at 2 wt %* *Palmitic Acid Methyl Ester (C16:0) at 6 wt %* *Palmitoleic Acid Methyl Ester (C16:1) at 2 wt %* *Heptadecanoic Acid Methyl Ester (C17:0) at 2 wt %* *cis-10-Heptadecenoic Acid Methyl Ester (C17:1) at 2 wt %* *Stearic Acid Methyl Ester (C18:0) at 4 wt %* *Oleic Acid Methyl Ester (C18:1n9c) at 4 wt %* *Elaidic Acid Methyl Ester (C18:1n9t) at 2 wt %* *Linoleic Acid Methyl Ester (C18:2n6c) at 2 wt %* *Linolelaidic Acid Methyl Ester (C18:2n6t) at 2 wt %* *γ-Linolenic Acid Methyl Ester (C18:3n6) at 2 wt %* *α-Linolenic Acid Methyl Ester (C18:3n3) at 2 wt %* *Arachidic Acid Methyl Ester (C20:0) at 4 wt %* *cis-11-Eicosenoic Acid Methyl Ester (C20:1n9) at 2 wt %* *cis-11,14-Eicosadienoic Acid Methyl Ester (C20:2) at 2 wt %* *cis-8,11,14-Eicosatrienoic Acid Methyl Ester (C20:3n6) at 2 wt %* *cis-11,14,17-Eicosatrienoic Acid Methyl Ester (C20:3n3) at 2 wt %* *Arachidonic Acid Methyl Ester (C20:4n6) at 2 wt %* *cis-5,8,11,14,17-Eicosapentaenoic Acid Methyl Ester (C20:5n3) at 2 wt %* *Heneicosanoic Acid Methyl Ester (C21:0) at 2 wt %* *Behenic Acid Methyl Ester (C22:0) at 4 wt %* *Erucic Acid Methyl Ester (C22:1n9) at 2 wt %* *cis-13,16-Docosadienoic Acid Methyl Ester (C22:2) at 2 wt %* *cis-4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester (C22:6n3) at 2 wt %* *Tricosanoic Acid Methyl Ester (C23:0) at 2 wt %* *Lignoceric Acid Methyl Ester (C24:0) at 4 wt %* *Nervonic Acid Methyl Ester (C24:1n9) at 2 wt %*	18919-1AMP
C8-C22 FAME Mix Neat mixture of 19 analytes, 100 mg total wt. *Caprylic Acid Methyl Ester (C8:0) at 1.9 wt %* *Capric Acid Methyl Ester (C10:0) at 3.2 wt %* *Lauric Acid Methyl Ester (C12:0) at 6.4 wt %* *Tridecanoic Acid Methyl Ester (C13:0) at 3.2 wt %* *Myristic Acid Methyl Ester (C14:0) at 3.2 wt %* *Myristoleic Acid Methyl Ester (C14:1) at 1.9 wt %* *Pentadecanoic Acid Methyl Ester (C15:0) at 1.9 wt %* *Palmitic Acid Methyl Ester (C16:0) at 13 wt %* *Palmitoleic Acid Methyl Ester (C16:1) at 6.4 wt %* *Heptadecanoic Acid Methyl Ester (C17:0) at 3.2 wt %* *Stearic Acid Methyl Ester (C18:0) at 6.5 wt %* *Oleic Acid Methyl Ester (C18:1n9c) at 19.6 wt %* *Elaidic Acid Methyl Ester (C18:1n9t) at 2.6 wt %* *Linoleic Acid Methyl Ester (C18:2n6c) at 13 wt %* *Linolenic Acid Methyl Ester (C18:3) at 6.4wt %* *Arachidic Acid Methyl Ester (C20:0) at 1.9 wt %* *cis-11-Eicosenoic Acid Methyl Ester (C20:1n9) at 1.9 wt %* *Behenic Acid Methyl Ester (C22:0) at 1.9 wt %* *cis-13-Docosanoic Acid Methyl Ester (C22:1) at 1.9 wt %*	18920-1AMP

Chemical Standards (Contd.)

Description	Cat. No.
C14-C22 FAME Mix Neat mixture of 10 analytes, 100 mg total wt. *Myristic Acid Methyl Ester (C14:0), 4% w/w* *Palmitic Acid Methyl Ester (C16:0), 10% w/w* *Stearic Acid Methyl Ester (C18:0), 6% w/w* *Oleic Acid Methyl Ester (C18:1n9c), 25% w/w* *Elaidic Acid Methyl Ester (C18:1n9t), 10% w/w* *Linoleic Acid Methyl Ester (C18:2n6c), 34% w/w* *Linolelaidic Acid Methyl Ester (C18:2n6t), 2% w/w* *Linolenic Acid Methyl Ester (C18:3), 5% w/w* *Arachidic Acid Methyl Ester (C20:0), 2% w/w* *Behenic Acid Methyl Ester (C22:0), 2% w/w*	18917-1AMP
C18-C20 FAME Mix Neat mixture of 6 analytes, 100 mg total wt. *Stearic Acid Methyl Ester (C18:0), 10% w/w* *Oleic Acid Methyl Ester (C18:1n9c), 20% w/w* *Elaidic Acid Methyl Ester (C18:1n9t), 20% w/w* *Linoleic Acid Methyl Ester (C18:2n6c), 20% w/w* *Linolelaidic Acid Methyl Ester (C18:2n6t), 20% w/w* *Arachidic Acid Methyl Ester (C20:0), 10% w/w*	18916-1AMP
Grain Fatty Acid Methyl Ester Mix 10 mg/mL (total wt.) in methylene chloride, 1 mL *Caprylic Acid Methyl Ester (C8:0), 1.9 wt. %* *Capric Acid Methyl Ester (C10:0), 3.2 wt. %* *Lauric Acid Methyl Ester (C12:0), 6.4 wt. %* *Tridecanoic Acid Methyl Ester (C13:0), 3.2 wt. %* *Myristic Acid Methyl Ester (C14:0), 3.2 wt. %* *Myristoleic Acid Methyl Ester (C14:1n9c), 1.9 wt. %* *Pentadecanoic Acid Methyl Ester (C15:0), 1.9 wt. %* *Palmitic Acid Methyl Ester (C16:0), 13.0 wt. %* *Palmitoleic Acid Methyl Ester (C16:1n9c), 6.4 wt. %* *Heptadecanoic Acid Methyl Ester (C17:0), 3.2 wt. %* *Stearic Acid Methyl Ester (C18:0), 6.5 wt. %* *Oleic Acid Methyl Ester (C18:1n9c), 19.6 wt. %* *Elaidic Acid Methyl Ester (C18:1n9t), 2.6 wt. %* *Linoleic Acid Methyl Ester (C18:2n6c), 13.0 wt. %* *α-Linolenic Acid Methyl Ester (C18:3n3), 6.4 wt. %* *Arachidic Acid Methyl Ester (C20:0), 1.9 wt. %* *cis-11-Eicosenoic Acid Methyl Ester (C20:1c), 1.9 wt. %* *Behenic Acid Methyl Ester (C22:0), 1.9 wt. %* *Erucic Acid Methyl Ester (C22:1n9), 1.9 wt. %*	47801

Blood Assessment Kits

Monitoring a patient's fatty acid profile is an important step in accurately managing wellness, allowing the health provider to verify the adherence to and effectiveness of a dietary strategy. Quick and accurate results are desirable so that any necessary changes to the dietary strategy can be made in a timely manner. It has been shown that blood samples collected as a small drop from the fingertip can be analyzed to provide sufficient data for such as assessment. (13)



Sigma-Aldrich offers convenient kits for the collection of blood drops, their storage/shipment, and processing to prepare samples for fatty acid analysis via gas chromatography. One kit is designed for collection and subsequent storage/shipment. The other kit is designed for derivatization of the fatty acids in the blood prior to GC analysis. Combined, these kits allow efficient sample collection and processing for quick compilation of analytical information on the fatty acid content in blood samples. They are tools that care providers can use in the development and application of adequate dietary strategies for their patients.

The Blood Collection Kit includes blood collection dipsticks, desiccant packs, foil-barrier ziplock bags, 50 mL BHT solution, and complete instructions. The Derivatization Kit includes a 1.25 M methanolic HCl solution, a saturated KCl solution, distilled water, and a working instruction sheet.

Description	Cat. No.
Blood Collection Kit, enough supplies for 100 tests	11312
Derivatization Kit, enough supplies for 100 tests	05904

GC Columns by Phase

Looking for information or specifications for a particular phase? This section provides application, USP code, polymer, and temperature limit information in addition to catalog numbers. (12) Where two maximum temperatures are listed (such as 200/220 °C), the first is for isothermal oven analyses, whereas the second is for oven temperature programmed analyses. Where only one maximum temperature is listed, it can be used for either isothermal or temperature programmed oven analyses.

This section is organized primarily in order of increasing phase polarity to assist in phase selection when performing method development. To learn more about any phases listed, or to inquire about a phase not listed, contact Technical Service at 800-359-3041 (US and Canada only), 814-359-3041, or at ***techservice@sial.com***

Equity-1

- **Application:** This column is designed for applications where a non-polar column is required. Analytes will be separated primarily according to boiling point.
- **USP Code:** This column meets USP G1, G2, and G9 requirements.
- **Polymer:** Bonded; poly(dimethylsiloxane)
- **Temperature Limits:**
  -60 °C to 325/350 °C for 0.10 - 0.32 mm I.D.
  -60 °C to 300/320 °C for 0.53 mm I.D. (≤1.5 µm)
  -60 °C to 260/280 °C for 0.53mm I.D. (>1.5 µm)

Description	Cat. No.
15 m x 0.10 mm I.D., 0.10 µm	28039-U
12 m x 0.20 mm I.D., 0.33 µm	28041-U
25 m x 0.20 mm I.D., 0.33 µm	28042-U
10 m x 0.20 mm I.D., 1.20 µm	28043-U
30 m x 0.25 mm I.D., 0.10 µm	28044-U
15 m x 0.25 mm I.D., 0.25 µm	28045-U
30 m x 0.25 mm I.D., 0.25 µm	28046-U
60 m x 0.25 mm I.D., 0.25 µm	28047-U
15 m x 0.25 mm I.D., 1.00 µm	28048-U
30 m x 0.25 mm I.D., 1.00 µm	28049-U
60 m x 0.25 mm I.D., 1.00 µm	28050-U
100 m x 0.25 mm I.D., 1.00 µm	28052-U
30 m x 0.32 mm I.D., 0.10 µm	28053-U
15 m x 0.32 mm I.D., 0.25 µm	28054-U
30 m x 0.32 mm I.D., 0.25 µm	28055-U
60 m x 0.32 mm I.D., 0.25 µm	28056-U
30 m x 0.32 mm I.D., 1.00 µm	28057-U
60 m x 0.32 mm I.D., 1.00 µm	28058-U
100 m x 0.32 mm I.D., 1.00 µm	28060-U
30 m x 0.32 mm I.D., 2.00 µm	28061-U
30 m x 0.32 mm I.D., 5.00 µm	28062-U
60 m x 0.32 mm I.D., 5.00 µm	28063-U
15 m x 0.53 mm I.D., 0.10 µm	28064-U
30 m x 0.53 mm I.D., 0.10 µm	28065-U
15 m x 0.53 mm I.D., 0.50 µm	28067-U
30 m x 0.53 mm I.D., 0.50 µm	28068-U
15 m x 0.53 mm I.D., 1.00 µm	28069-U
30 m x 0.53 mm I.D., 1.00 µm	28071-U
15 m x 0.53 mm I.D., 1.50 µm	28072-U
30 m x 0.53 mm I.D., 1.50 µm	28073-U
60 m x 0.53 mm I.D., 1.50 µm	28074-U
15 m x 0.53 mm I.D., 3.00 µm	28075-U
30 m x 0.53 mm I.D., 3.00 µm	28076-U
60 m x 0.53 mm I.D., 3.00 µm	28077-U
15 m x 0.53 mm I.D., 5.00 µm	28079-U
30 m x 0.53 mm I.D., 5.00 µm	28081-U
60 m x 0.53 mm I.D., 5.00 µm	28082-U

Nukol

- **Application:** The incorporation of acid functional groups into the phase lends an acidic character to this column, useful for analyses of volatile acidic compounds. Difficult to analyze carboxylic acids (free fatty acids) can be analyzed with excellent peak shape and minimal adsorption.
- **USP Code:** This column meets USP G25 and G35 requirements.
- **Polymer:** Bonded; acid-modified poly(ethylene glycol)
- **Temperature Limits:**
  60 °C to 200/220 °C

Description	Cat. No.
15 m x 0.25 mm I.D., 0.25 µm	24106-U
30 m x 0.25 mm I.D., 0.25 µm	24107
60 m x 0.25 mm I.D., 0.25 µm	24108
15 m x 0.32 mm I.D., 0.25 µm	24130
30 m x 0.32 mm I.D., 0.25 µm	24131
60 m x 0.32 mm I.D., 0.25 µm	24132
15 m x 0.32 mm I.D., 1.00 µm	24206-U
30 m x 0.32 mm I.D., 1.00 µm	24207
60 m x 0.32 mm I.D., 1.00 µm	24208
15 m x 0.53 mm I.D., 0.50 µm	25326
30 m x 0.53 mm I.D., 0.50 µm	25327
60 m x 0.53 mm I.D., 0.50 µm	25386
30 m x 0.53 mm I.D., 1.00 µm	25357

Omegawax

- **Application:** This column allows highly reproducible analyses of fatty acid methyl esters (FAMEs), specifically omega 3 and omega 6 groups. It is tested to ensure reproducible FAME equivalent chain length (ECL) values and resolution of key components. This column is specified in AOAC Method 991.39 and AOCS Method Ce 1b-89.
- **USP Code:** This column meets USP G16 requirements.
- **Polymer:** Bonded; poly(ethylene glycol)
- **Temperature Limits:**
  50 °C to 280 °C

Description	Cat. No.
15 m x 0.10 mm I.D., 0.10 µm	23399-U
30 m x 0.25 mm I.D., 0.25 µm	24136
30 m x 0.32 mm I.D., 0.25 µm	24152
30 m x 0.53 mm I.D., 0.50 µm	25374

SP-2560

- **Application:** This highly polar biscyanopropyl column was specifically designed for the detailed separation of geometric (cis/trans) isomers of fatty acid methyl esters (FAMEs). It is extremely effective for FAME isomer applications. This column is specified in AOAC Method 996.06 and AOCS Method Ce 1h-05.
- **USP Code:** This column meets USP G5 requirements.
- **Polymer:** Non-bonded; poly(biscyanopropyl siloxane)
- **Temperature Limits:**
  Subambient to 250 °C

Description	Cat. No.
75 m x 0.18 mm I.D., 0.14 µm	23348-U
100 m x 0.25 mm I.D., 0.20 µm	24056
100 m x 0.25 mm I.D., 0.20 µm*	23362-U

* Wound onto a 5" cage to fit an Agilent® 6850 GC.

SP-2380

- **Application:** A highly polar cyanosiloxane column commonly used for separation of geometric (cis/trans) fatty acid methyl ester (FAME) isomers as a group. Also useful when a highly polar general purpose column with good thermal stability is required.
- **USP Code:** This column meets USP G48 requirements.
- **Polymer:** Stabilized; poly(90% biscyanopropyl/10% cyanopropylphenyl siloxane)
- **Temperature Limits:**
  Subambient to 275 °C

Description	Cat. No.
15 m x 0.25 mm I.D., 0.20 µm	24109
30 m x 0.25 mm I.D., 0.20 µm	24110-U
60 m x 0.25 mm I.D., 0.20 µm	24111
100 m x 0.25 mm I.D., 0.20 µm	24317
30 m x 0.32 mm I.D., 0.20 µm	24116-U
60 m x 0.32 mm I.D., 0.20 µm	24117
30 m x 0.53 mm I.D., 0.20 µm	25319

SLB-IL100

- **Application:** This ionic liquid phase has a polarity/selectivity roughly equivalent to that of the TCEP phase, higher than any of the polysiloxane polymer and polyethylene glycol phases. The combination of high polarity/selectivity, low bleed, and a maximum temperature of 230 °C results in a column very effective for analyses of FAMEs, aromatics, and PCB congeners.
- **USP Code:** None.
- **Polymer:** Non-bonded; 1,9-di(3-vinyl-imidazolium) nonane bis(trifluoromethyl) sulfonyl imidate
- **Temperature Limits:**
  Subambient to 230 °C

Description	Cat. No.
15 m x 0.10 mm I.D., 0.08 µm	28882-U
20 m x 0.18 mm I.D., 0.14 µm	28883-U
30 m x 0.25 mm I.D., 0.20 µm	28884-U
60 m x 0.25 mm I.D., 0.20 µm	28886-U
30 m x 0.32 mm I.D., 0.26 µm	28887-U
60 m x 0.32 mm I.D., 0.26 µm	28888-U

References

1. A. Ascherio, W. Willett, "Health Effects of Trans Fatty Acids" Am. J. Clin. Nutr. (1997) 66 (supplement), 1006S-1010S.
2. S. Stender, J. Dyerberg, "Influence of Trans Fatty Acids on Health" Annals of Nutrition and Metabolism (2004) 48 (2), 61-66.
3. American Heart Association Web Page, http://www.americanheart.org/presenter.jhtml?identifier= 1728 (accessed Jan. 4, 2006).
4. 21 CFR Part 101, "Food Labeling: Trans Fatty Acids in Nutrition Labeling" Federal Register (July 11, 2003) Volume 68, Number 133, http://www.cfsan.fda.gov/~lrd/fr03711a.html (accessed Jan. 4, 2006).
5. W. W. Christie, "Gas Chromatography and Lipids" The Lipid Library, http://www.lipidlibrary.co.uk/GC_lipid/gc_lip.html (accessed Jun 26, 2008).
6. W. W. Christie, "Why I Dislike Boron Trifluoride-Methanol" Lipid Technology (1994) 6, 66-68.
7. AOCS Method Ce 1-62, "Fatty Acid Composition by Gas Chromatography" AOCS Official Methods (2005) American Oil Chemists Society.
8. AOAC Method 991.39, "Fatty Acids in Encapsulated Fish Oils and Fish Oil Methyl and Ethyl Esters" Official Methods of Analysis, 18th Edition (on-line) Association of Official Analytical Chemists, Inc.
9. AOCS Method Ce 1b-89, "Fatty Acid Composition by GLC Marine Oils" AOCS Official Methods (2005) American Oil Chemists Society.
10. AOAC Method 996.06, "Fat (Total, Saturated, and Unsaturated) in Foods" Official Methods of Analysis, 18th Edition (on-line) Association of Official Analytical Chemists, Inc.
11. AOCS Method Ce 1h-05, "Determination of cis-, trans-, Saturated, Monounsaturated and Polyunsaturated Fatty Acids in Vegetable or Non-ruminant Animal Oils and Fats by Capillary GLC" AOCS Official Methods (2005) American Oil Chemists Society.
12. USP, "Chromatographic Reagents" United States Pharmacopeia 31 / National Formulary 26, First Supplement (August 1, 2008) 3596-3598.
13. F. Marangoni, C. Colombo, C. Galli, "A Method for the Direct Evaluation of the Fatty Acid Status in a Drop of Blood from a Fingertip in Humans: Applicability to Nutritional and Epidemiological Studies" Anal. Biochem. (2004) 326, 267-272.

Product Literature

The following list of Sigma-Aldrich/Supelco literature provides additional product information than what is presented in this brochure. To obtain any of these literature pieces at no-charge, either visit our web site at *sigma-aldrich.com* or contact Supelco Technical Service: 800-359-3041 (US and Canada only), 814-359-3041, or at ***techservice@sial.com***

Title	Identification
GC Columns	
GC Column Selection Guide	T407133 KCX
Analyzing Fatty Acids by Capillary GC	T110855 AYC
37-Component FAME Mix on Four Capillary Columns	T196907 AZC
Fast GC Brochure	T407096 JTW
Capillary GC Troubleshooting Guide	T112853 AIP
GC-Related	
GC Accessories and Gas Purification/Management	T407103 JWE
Molded Thermogreen™ LB-2 Septa	T407082 JQV
Selecting the Appropriate Inlet Liner (Poster)	T404081 HCH
Gas Management Systems for GC	T196898 AYW
Gas Generators Brochure	T407110 JXP
Syringes Brochure	T406108 JCS
Vials Brochure	IXH
Chemical Standards	
Fluka Analytical Reagents & Standards Catalog	003
SPE Tubes	
Discovery Ag-Ion SPE for cis/trans FAME Fractionation	T406062 IRV
Supelco Solid Phase Extraction Products	T402150 FEB
Derivatization Reagents	
Derivatization Reagents Brochure	T407138 KDI
BCl_3-Methanol (12% w/w)	T496123 BAX
BF_3-Methanol (10% w/w)	T496125 BAZ
BF_3-Butanol (10% w/w)	T496124 BAY
Methanolic Base (0.5N)	T497007 BEG
Methanolic HCl (0.5N and 3N)	T497099 BIV
Methanolic H_2SO_4 (10% v/v)	T497018 BDO

Additional Reading

Consult these references, written by experts and researchers, to learn more about the many facets of fatty acids, FAMEs, and their analysis.

1. William W. Christie, "Lipid Analysis: Isolation, Separation, Identification and Structural Analysis of Lipids" Third Edition (2003) The Oily Press, ISBN 0-9531949-5-7.
2. William W. Christie, "Gas Chromatography and Lipids" The Lipid Library, http://www.lipidlibrary.co.uk/GC_lipid/gc_lip.html.
3. Frank D. Gunstone, "Lipids for Functional Foods and Nutraceuticals" (2003) The Oily Press, ISBN 0-9531949-3-0.
4. Daniel R. Knapp, "Handbook of Analytical Derivatization Reactions" (1979) Wiley, ISBN 978-0-471-03469-8.
5. Karl Blau and John M. Halket, "Handbook of Derivatives for Chromatography" Second Edition (1993) Wiley, ISBN 978-0-471-92699-3.
6. Harold McNair and James Miller, "Basic Gas Chromatography" (1998) Wiley, ISBN 0-471-17261-8.
7. David Grant, "Capillary Gas Chromatography" (1996) Wiley, ISBN 0-471-95377-6.
8. Robert L. Grob and Eugene F. Barry, "Modern Practices of Gas Chromatography" Fourth Edition (2004) Wiley, ISBN 0-471-22983-0.
9. Eugene F. Barry and Robert L. Grob, "Columns for Gas Chromatography: Performance and Selection" (2007) Wiley, ISBN 978-0-471-74043-8.
10. Konrad Grob, "Split and Splitless Injection in Capillary GC" (1993) Hüthig, ISBN 3-7785-2151-9.
11. Dean Rood, "A Practical Guide to the Care, Maintenance, and Troubleshooting of Capillary Gas Chromatographic Systems" (1991) Hüthig, ISBN 3-7785-1898-4.

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SIGMA-ALDRICH DE ARGENTINA S.A.
Free Tel: 0810 888 7446
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SIGMA-ALDRICH PTY LTD.
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Free Fax: 1800 800 096
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Free Fax: 0800 031 052
Tel: (+33) 474 82 28 00
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Free Tel: 0800 51 55 000
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Tel: (+49) 89 6513 0
Fax: (+49) 89 6513 1160

Greece
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Fax: (+30) 210 994 3831

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SIGMA-ALDRICH Kft
Ingyenes telefonszám: 06 80 355 355
Ingyenes fax szám: 06 80 344 344
Tel: (+36) 1 235 9055
Fax: (+36) 1 235 9050

India
SIGMA-ALDRICH CHEMICALS
PRIVATE LIMITED
Telephone
Bangalore: (+91) 80 6621 9600
New Delhi: (+91) 11 4358 8000
Mumbai: (+91) 22 2570 2364
Hyderabad: (+91) 40 4015 5488
Fax
Bangalore: (+91) 80 6621 9650
New Delhi: (+91) 11 4358 8001
Mumbai: (+91) 22 2579 7589
Hyderabad: (+91) 40 4015 5466

Ireland
SIGMA-ALDRICH IRELAND LTD.
Free Tel: 1800 200 888
Free Fax: 1800 600 222
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Fax: + 353 (0) 402 20375

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SIGMA-ALDRICH ISRAEL LTD.
Free Tel: 1 800 70 2222
Tel: (+972) 8 948 4100
Fax: (+972) 8 948 4200

Italy
SIGMA-ALDRICH S.r.l.
Numero Verde: 800 827018
Tel: (+39) 02 3341 7310
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Japan
SIGMA-ALDRICH JAPAN K.K.
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Fax: (+81) 3 5796 7315

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SIGMA-ALDRICH KOREA
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Tel: (+82) 31 329 9000
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Fax: (+41) 81 755 2815

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St. Louis, Missouri 63178
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