Analyzing Fatty Acids by Capillary Gas Chromatography

Described here are capillary gas chromatographic analyses of volatile and nonvolatile fatty acids, including cis/trans isomers, in free form and as methyl esters. Choose from conditions under which these compounds are eluted by chain length and degree of unsaturation, or by boiling point. Columns for these analyses are listed

Key Words:
● fatty acids ● fatty acid methyl esters ● volatile fatty acids
● carboxylic acids ● food acids

Fatty Acids Analyses Summarized

Analysis	Column
C2-C20+ Free Acids	Nukol
C10-C20+ FAMEs:	
·by chain length/degree of unsaturation	Omegawax 250, Omegawax 320, SUPELCOWAX 10
·to resolve groups of *cis* and *trans* isomers, double bond positional isomers	SP-2380, SP-2340, SP-2330
·for maximum resolution of *cis* or *trans* isomers	SP-2560
·for boiling point elution patterns (e.g., bacterial acid FAMEs)	SPB-1

Analyzing Free Acids Without Peak Tailing

In their underivatized forms, acidic compounds such as fatty (carboxylic) acids are difficult to quantify accurately by capillary GC. Because these highly polar compounds tend to form hydrogen bonds, they are often adsorbed by the column. As a result, they elute as tailing or poorly resolved peaks. For some compounds, adsorption can be irreversible. Although nonbonded phase capillary columns have been treated in various ways to minimize adsorption of acidic samples, treated bonded phase columns have only recently become available.

Nukol™ acidic, bonded phase capillary columns are ideal for analyses of free fatty acids (and for glycols, phenols, alcohols, and many other acidic polar compounds, as well). Such compounds are eluted from Nukol columns as sharp, symmetric peaks (1): Figure A shows the separation of short chain (C2-C7) free fatty acids on a 30m x 0.25mm ID Nukol column. The isothermal analysis is completed in less than 10 minutes, and the peaks are sharp and symmetric.

Figure A. Short Chain Free Fatty Acids Separated in Less Than 10 Minutes

Column: **Nukol, 30m x 0.25mm ID, 0.25µm film**
Cat. No.: **24107**
Oven: 185°C
Carrier: helium, 20cm/sec
Det.: FID
Inj.: 1µL Volatile Acids Mix (Cat. No. 46975-U), split 100:1

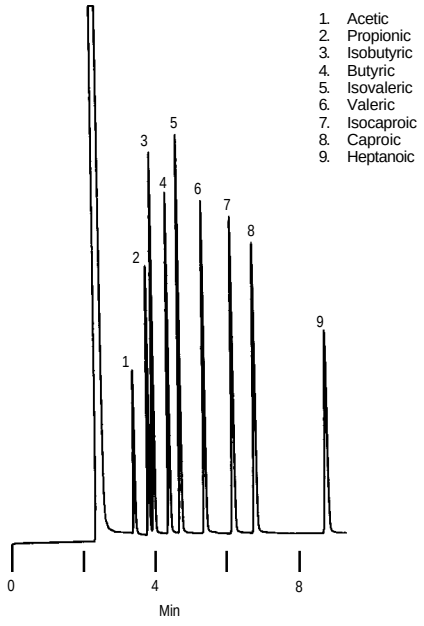

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Figure B shows C2-C20 free acids analyzed on a 15m x 0.53mm ID Nukol column. The volatile C2-C7 acids are well resolved. Minor tailing of these peaks is caused by the combination of solvent elution effects and the high carrier gas flow rate (30mL/minute) used to elute the longer chain acids. Note that the eicosanoic acid (C20) peak is symmetrical, even though this acid is eluted at a temperature 140°C below its boiling point. In addition, the analysis is completed in well under 20 minutes. Analyzing the acids in free form saves the time and costs (and eliminates potential errors) involved in sample methylation.

The high carrier gas flow rate is an important factor in this analysis. It enables the longer chain acids, even eicosanoic acid, to be eluted as symmetric peaks at low temperatures. Furthermore, because 0.53mm ID Nukol columns accept high flow rates, you can use them in packed column instruments with injectors and detectors

Figure B. Long Chain Free Acids Eluted Without Tailing

Column: **Nukol, 15m x 0.53mm ID, 0.50µm film**
Cat. No.: **25326**
Oven: 100°C to 220°C at 10°C/min
Carrier: helium, 30mL/min
Det.: FID
Inj.: 0.5µL acids mixture (25-400ng each component), direct injection

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

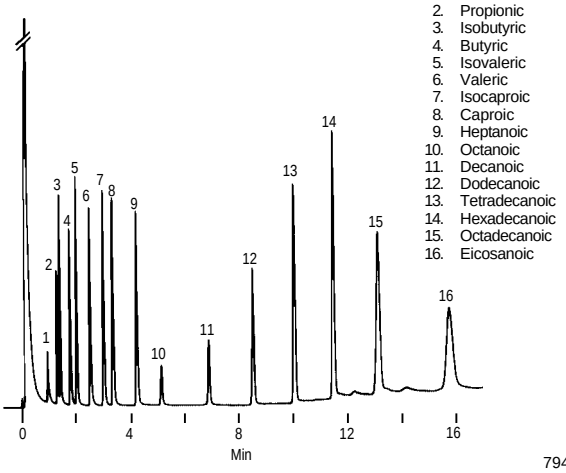

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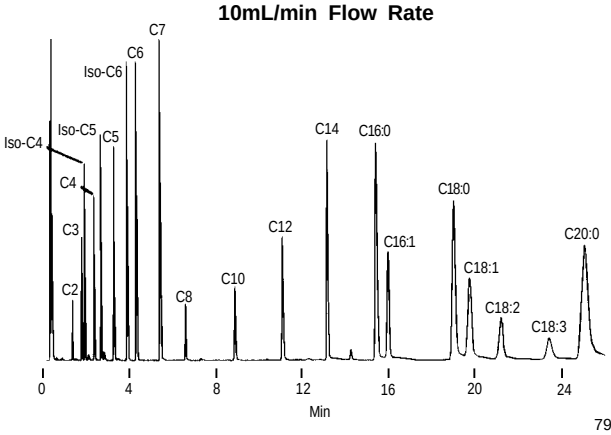
Figure C. C2-C22 Free Fatty Acids

Column: **Nukol, 15m x 0.53mm ID, 0.50µm film**
Cat. No.: **25326**
Oven: 110°C to 220°C at 8°C/min
Carrier: helium
Det.: FID
Inj.: 0.20µL of a 100µg/µL mixture, direct injection

10mL/min Flow Rate



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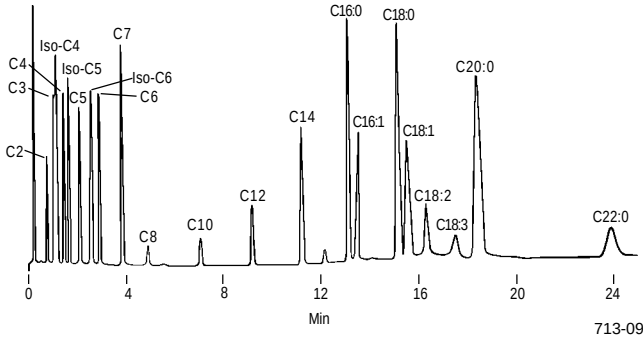
20mL/min Flow Rate
Sample Range Increased without Prolonging Analysis



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designed for packed columns, as well as in dedicated capillary instruments. (For more information on using wide bore capillary columns in packed column instruments, refer to our current catalog.)

Figure C shows that a Nukol column will also resolve numerous isomers, such as nC4-nC6 acids from corresponding iso forms, and, especially, saturated from unsaturated acids (e.g., C16:0 from C16:1, C18:0/18:1/18:2/18:3). The isomers elute in order by their degree of unsaturation, from saturated to most unsaturated (e.g., C18:0→C18:3). Because the 0.53mm ID column accepts high carrier gas flows, it can be used to extend the analysis to C22:0 in the same amount of time, with no noticeable loss in resolution. If you are using a sample preparation method that calls for methylation, a Nukol column also will separate these compounds as methyl esters, in the same elution order as the free acids and with equally satisfactory results.

Figure D shows saponified, then acidified, food oil samples analyzed on a Nukol column. The fatty acid isomers are well resolved and can be readily quantified. The vegetable source and animal source materials are easily distinguished.

If you are now using a nonbonded SP™-1000 or other PEG-type, moderately polar capillary column to analyze underivatized fatty acids, you will find separations are very similar on a Nukol column. A Nukol column's ability to be rinsed with solvents is a valuable added feature, however, because many samples deposit nonvolatile components in the inlet end of the column. These deposits act as active sites, adsorbing the acids in later samples. In Nukol columns, these deposits can be removed when they begin to affect the analyses. Thus, column life is prolonged. Furthermore, the bonded Nukol phase is unaffected by repeated injections of large volumes of solvents. This includes water, in which many acidic samples are dissolved.

Analyzing C14-C20+ Fatty Acids as Methyl Esters

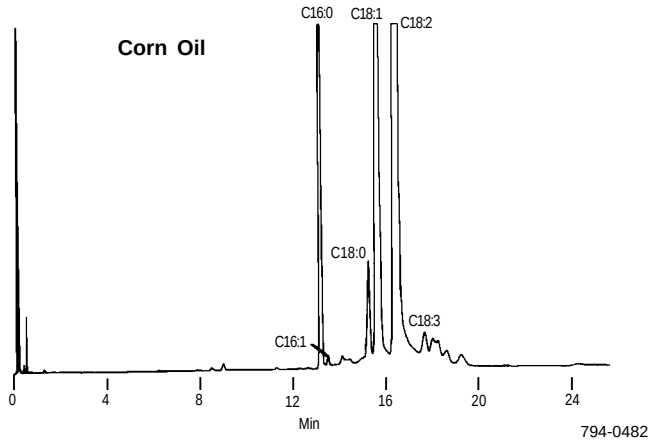
Moderately Polar, Polyethylene Glycol Phase Columns

Analysts evaluating vegetable, animal organ, or marine fish oils must monitor even-numbered, straight chain fatty acid methyl esters (FAMEs) containing single and multiple *cis* double bonds. To prevent analytical errors in identifying and quantifying these esters, the GC column used should elute the compounds primarily by carbon chain length and secondarily by the number of double bonds (2). There should be minimal overlap in elution order among FAMEs having different chain lengths (all C18 esters should elute before the C20 esters, etc.). Analysts also have recognized the importance of using equivalent chain length (ECL) values (3,4) to predict the identity of fatty acids in natural samples. Because the *trans* isomer content of such samples is negligible, group separations of *cis* isomers from *trans* isomers are not an important consideration.

Polyethylene glycol (PEG) phase capillary columns (e.g., CARBOWAX® 20M PEG columns) resolve these compounds with little or no overlap in the elution order of FAMEs of different carbon chain length (5). However, column to column variability in performance among PEG columns has made it difficult to rely on equivalent chain length values for identifying sample components, particularly when making interlaboratory comparisons. Peak coelutions also are often encountered when using PEG columns for FAMEs analyses. One of the common coelutions, C24:0 with C22:6n3, is a major concern because the concentration

Figure D. Saponified Fatty Acids from Food Oils

Column: **Nukol, 15m x 0.53mm ID, 0.50µm film**
Cat. No.: **25326**
Oven: 110°C to 220°C at 8°C/min
Carrier: helium, 20mL/min
Det.: FID
Inj.: 0.20µL of saponified mixed acids (approx. 20µg), direct injection



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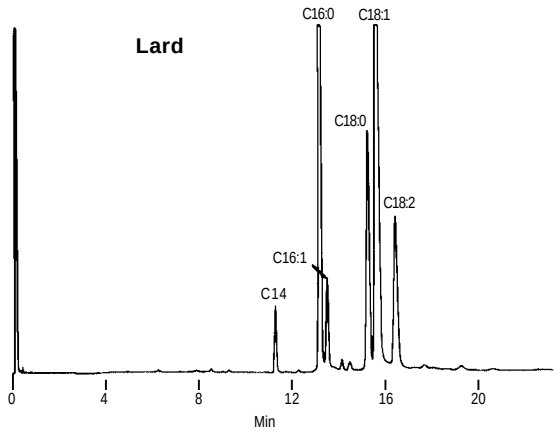

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of C22:6n3 (docosahexaenoic acid, DHA) is monitored in Association of Official Analytical Chemists and American Oil Chemists Society methods (6,7). Other common coelutions are C21:5n3 with the C23:0 internal standard used in many quantitative FAMEs analyses, and C22:6n3 with the C24:1n9 FAME which is being proposed as an internal standard.

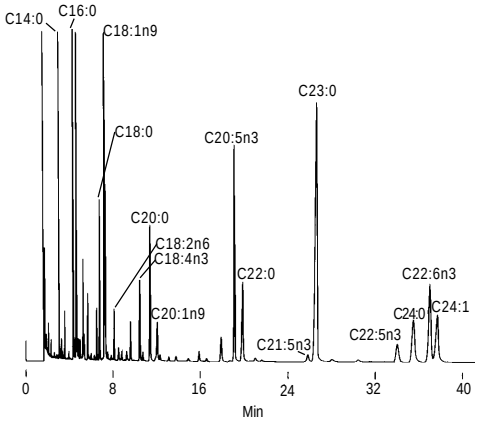
Omegawax™ 250 and Omegawax 320 bonded PEG phase capillary columns are tested to ensure consistent equivalent chain length values from column to column, with minimal or no overlap among carbon chains of differing length. They are specifically prepared and tested for analyses of omega-3 and omega-6 fatty acid methyl esters, as described in AOAC and AOCS methods (6,7) and in pending revisions specifying use of capillary columns (8).

To ensure reproducible column performance, we have optimized the polarity of Omegawax columns for FAMEs analyses and maximized resolution between the C23:0 (internal standard) and C21:5n3 FAMEs, while maintaining good resolution among other important FAMEs. Figure E, an analysis of our test mixture for Omegawax columns, is representative of column performance in fish oil FAMEs analyses. For each column there is good separation between C23:0 and C21:5n3 and near baseline resolution between C24:0, C22:6n3 (DHA), and C24:1. Resolution of the other FAMEs also is very good. There is only one overlap of even carbon numbered FAMEs – C24:0 elutes before C22:6n3.

Figure E. Omega-3 FAMEs on Omegawax Columns

Omegawax 250 Column

Column: **Omegawax 250, 30m x 0.25mm ID, 0.25µm film**
Cat. No.: **24136**
Oven: 205°C
Carrier: helium, 30cm/sec, set at 205°C
Det.: FID, 260°C
Inj.: 1µL of Omegawax Test Mix (25mg FAMEs/mL hexane), split 100:1, 250°C



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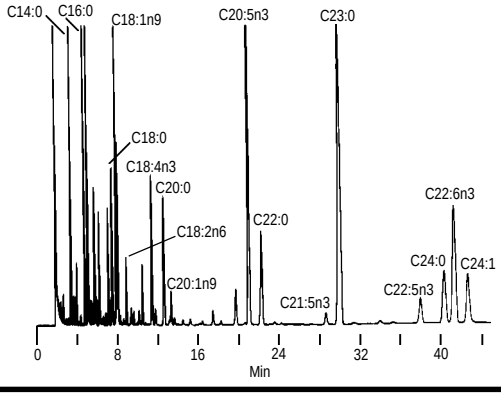
Omegawax 320 Column

Column: **Omegawax 320, 30m x 0.32mm ID, 0.25µm film**
Cat. No.: **24152**
Oven: 200°C
Carrier: helium, 25cm/sec, set at 200°C (135-140cc/min split vent flow, 30cc/min nitrogen make-up gas)
Det.: FID, 260°C
Inj.: 1µL of Omegawax Test Mix (50mg FAMEs/mL hexane), split 100:1, 250°C



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The test mix for Omegawax columns is based on menhaden oil for two reasons: menhaden oil contains most of the fatty acids commonly found in products comprised of omega-3 and omega-6 fatty acids, including a large amount of C20:5n3 (eicosapentaenoic acid, EPA) and C22:6n3 (DHA), and partially hydrogenated menhaden oil recently was given GRAS (generally recognized as safe) status by the US Food and Drug Administration (9) and can now be used as a food additive. We felt it would be beneficial to evaluate the column with a sample it will be routinely used to analyze.

To prepare the test mix, we fortify the levels of the C20, C22, and C24 saturated fatty acids normally found in small quantities in menhaden oil. This allows us to calculate ECL values (7). We also add C23:0 and C24:1 as internal standards.

Table 1 shows the reproducibility of Omegawax 320 column performance. As polarity markers, we monitored the equivalent chain length values for four highly unsaturated FAMEs. Even slight column to column differences in polarity will be revealed by these probes. The resolution value for the C18:0/C18:1n9 separation is

a measure of column efficiency cited in the AOAC and AOCS methods. The small standard deviations and percent relative standard deviations for ECL values for 55 columns (representing three production lots) reveal minimal variability, indicating column polarity is very consistent from column to column. The small standard deviation and percent relative standard deviation for C18:0/C18:1n9 resolution show very consistent column efficiency. Similar results are obtained from Omegawax 250 columns.

Cod liver oil contains large amounts of omega-3 fatty acids. Figure F1 shows an Omegawax 320 column provides good resolution of these various components and reveals differences in the relative ratios of key components, compared to the menhaden oil-based test mix. Like other marine fish oils, cod liver oil contains large amounts of EPA and DHA. In contrast to marine fish oils, land animal

Table 1. Omegawax 320 Columns Ensure Consistent Equivalent Chain Length Values*

	FAME / ECL Value				
	C18:4n3	C20:5n3	C21:5n3	C22:5n3	Resolution
Mean	19.64	21.81	22.84	23.80	3.99
Std. Dev.	0.01	0.01	0.01	0.01	0.11
% RSD	0.05	0.06	0.06	0.06	2.85

*Mean, standard deviation, and relative standard deviation for 55 columns from 3 production lots.

tissue oils contain a high percentage of omega-6 fatty acids. Figure F2 shows an analysis of a land animal organ extract containing significant amounts of C20:4n6 and C22:4n6 acids. The omega-3 and omega-6 series coexist in many samples, such as human blood (10, 11), human milk (12) and freshwater fish (13) lipids. These samples can also be analyzed on Omegawax 250 columns. As mentioned earlier, these naturally occurring mixtures consist primarily of *cis* isomers. Research at the Canadian Institute of Fisheries Technology has shown that when naturally occurring fatty acids are synthetically modified to form complex mixtures of *cis* and *trans* isomers (e.g., by heating), both a PEG phase column and a polar SP-2340 cyanosilicone phase column may be required to obtain the most information (14).

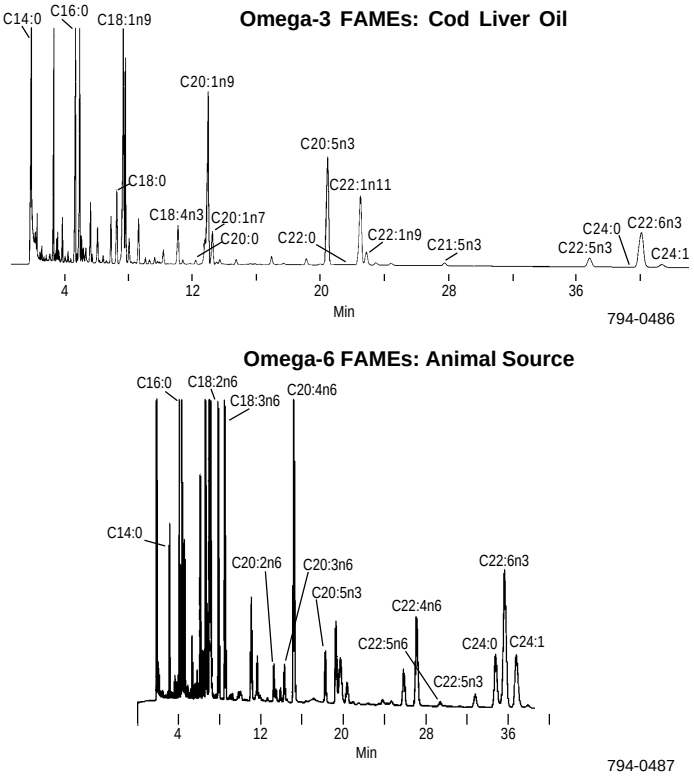
To demonstrate the performance of the Omegawax 250 column, we analyzed samples of catfish filet, brain, and liver (Figure G). The resolution provided by the Omegawax 250 column revealed a number of tissue to tissue differences in the amounts of specific FAMEs. Filet and liver, for example, contained higher amounts of methyl linoleate (C18:2n6) than did the brain. Also note the larger number of compounds eluted from the brain sample between C:16 and C18:2n6, and the lower amount of C20:5n3. More C22:6n3 was present in the brain and liver than in the filet.

The ability to identify such FAME profile differences makes the Omega 250 column an excellent tool for comparing the various tissues of the same fish or other organism. Similar results can be obtained in analyzing tissue between species.

SUPELCOWAX™ 10 bonded PEG phase columns also resolve even-numbered, straight chain FAMEs primarily by chain length and secondarily by the number of double bonds. SUPELCOWAX 10 columns are available in 0.20mm, 0.53mm, and, as custom products, 0.75mm ID, as well as in 0.25mm and 0.32mm ID. The wide bore columns (Figure H) have large sample capacity and enable you to monitor both highly concentrated FAMEs and trace FAMEs in a single analysis. They can also be used in instruments

Figure F. Omegawax 320 Column Resolves Omega-3 and Omega-6 FAMEs

Column: **Omegawax 320, 30m x 0.32mm ID, 0.25µm film**
Cat. No.: **24152**
Oven: 200°C
Carrier: helium, 25cm/sec, set at 200°C (135-140cc/min split vent flow, 30cc/min nitrogen make-up gas)
Det.: FID, 260°C
Inj.: 1µL hexane containing polyunsaturated FAMEs (50mg/mL), marine source (Cat. No. 4-7033) or animal source (Cat. No. 47015-U), split 100:1, 250°C

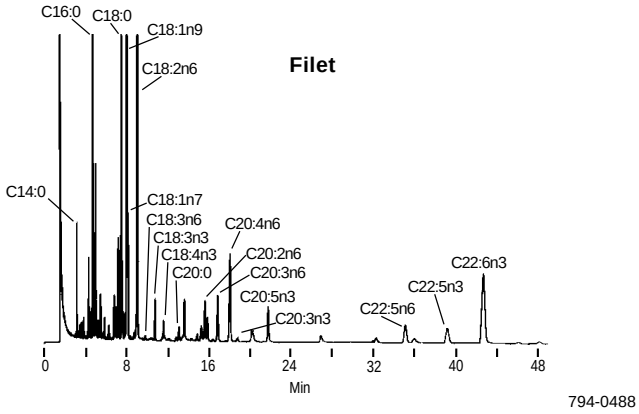


designed for packed columns. The 0.20mm ID columns provide maximum resolution of complex FAMEs mixtures.

Omegawax columns and SUPELCOWAX 10 columns have very good thermal stability. They can be used isothermally, to 280°C, to provide excellent, highly reproducible resolution of nonhydrogenated FAMEs. The thermally stable columns provide a wide temperature range within which a particular analysis can be performed without changes in the sample component elution order (15,16). With some comparable columns, deviation from a narrow temperature range makes peak shifts more likely. Thermally stable Omegawax columns and SUPELCOWAX 10 columns also have potentially long lifetimes. At the Canadian Institute of Fisheries Technology, Dr. Robert Ackman used a SUPELCOWAX 10 column continuously for more than 6 months, with no deterioration in performance (15,16).

Figure G. Significant Differences in the FAME Profiles of Catfish Tissues, Revealed by Using an Omegawax 250 Column

Column: **Omegawax 250, 30m x 0.25mm ID, 0.25µm film**
Cat. No.: **24136**
Oven: 205°C
Carrier: helium, 30cm/sec, set at 205°C
Det.: FID, 260°C
Inj.: 1µL of Omegawax Test Mix (25mg FAMEs/mL hexane), split 100:1, 250°C



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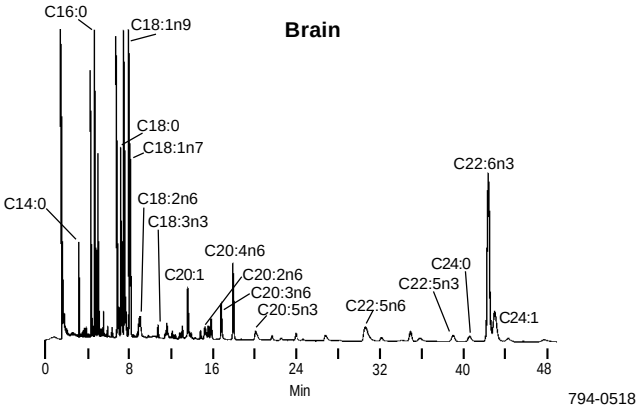

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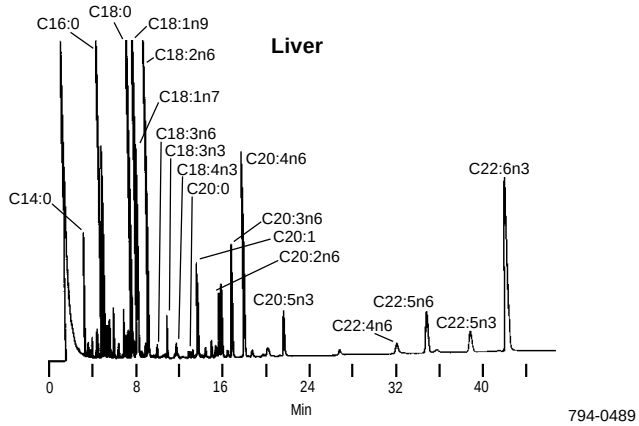

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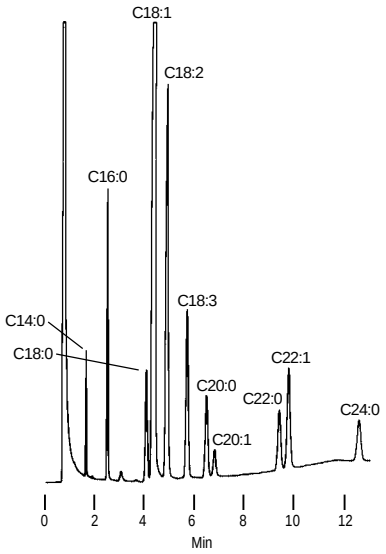
Figure H. Fatty Acid Methyl Esters on a Wide Bore SUPELCOWAX 10 Column (Temperature Programmed Analysis)

Column: **SUPELCOWAX 10, 30m x 0.75mm ID, 1.0µm film**
Cat. No.: **available on request; separations are similar on a 0.53mm ID column**
Oven: 210°C (4 min) to 240°C at 4°C/min
Carrier: helium, 50cm/sec
Det.: FID
Inj.: 0.1µL of diluted rapeseed oil reference mixture (1µg FAMEs)



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Polar, Cyanosilicone Phase Columns

Cyanosilicone stationary phases are among the most polar stationary phases available. The high polarity of these phases allows analysts to separate polar compounds, with close boiling points, that cannot be resolved by nonpolar or intermediate polarity phases. Thus, cyanosilicone phases can resolve groups of *cis, trans*, or olefinic positional isomers (Figure I). The *trans* isomers, in groups, elute before the *cis* isomers of the same chain length and degree of unsaturation. In more complex mixtures, double bond positional isomers can also be resolved. These columns are useful when you wish to separate isomer groups, or when an Omegawax or SUPELCOWAX 10 column does not provide the specific separation you need (14). Because separations on cyanosilicone phase columns depend on polar interactions as well as on boiling point, however, there will be crossover in elution sequence among FAMEs of different chain length and degree of unsaturation.

Most cyanosilicone phase columns, such as SP-2330 and SP-2340 columns, are nonbonded phase columns. Due to thermal rearrangement, they have a limited maximum temperature and lifespan. Compared to these columns, a stabilized (partially crosslinked) SP-2380 column provides increased temperature stability and longer life in these analyses. The 275°C upper temperature limit of SP-2380 columns is at least 25°C higher than that for other cyanosilicone phase columns. Thus, you can resolve higher molecular weight sample components without interference from column bleed. Figure J shows a temperature programmed analysis of methyl esters of fatty acids and tall oil rosin acids. The baseline is virtually flat over the entire temperature program.

The polarity of SP-2380 columns lies between that of SP-2330 and SP-2340 columns, and is closer to the latter (17). Because the three column types have similar polarity, an SP-2380 column can be used in place of either of the other columns without changing analytical conditions.

Figure I. *cis/trans* Isomers on a Cyanosilicone Phase Column

Column: **SP-2330, 30m x 0.25mm ID, 0.20µm film**
Cat. No.: **24019**
Oven: 190°C
Carrier: helium, 20cm/sec set at 190°C
Det.: FID, 250°C
Inj.: 1µL of diluted rapeseed oil reference mixture (10µg FAMEs in methylene chloride), split 100:1, 250°C

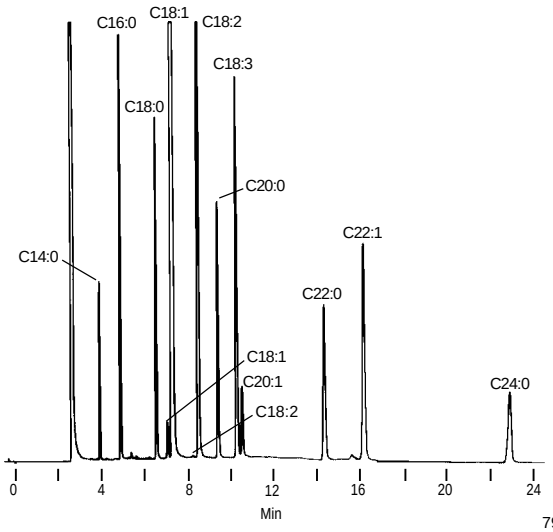

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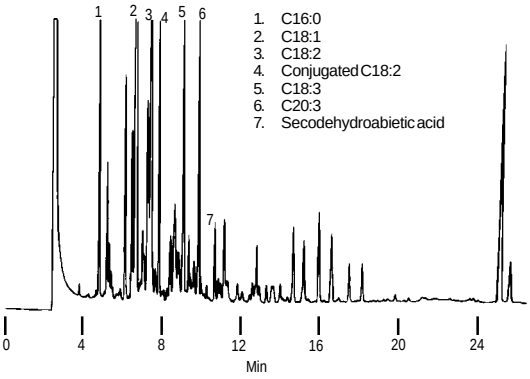
Figure J. Stable Baseline During Temperature Programmed Analysis

Column: **SP-2380, 30m x 0.25mm ID, 0.20µm film**
Cat. No.: **24110-U**
Oven: 170°C to 260°C at 4°C/min, hold 4 min
Carrier: helium, 20cm/sec
Det.: FID
Inj.: 2µL methyl chloride containing tall oil methyl esters, split 100:1



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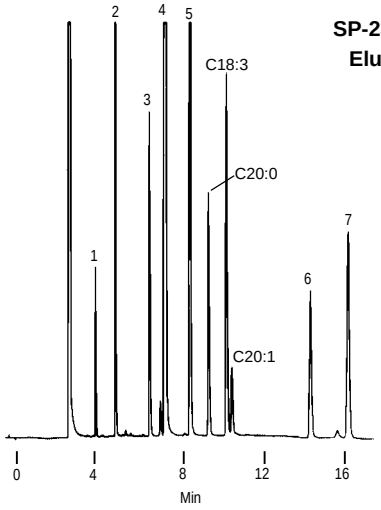
To demonstrate the minor differences in analyses performed on these columns, we analyzed a fatty acid methyl ester (FAME) standard, rapeseed oil, on each of the three cyanosilicone phases. Temperature and other conditions were held constant. The separations are compared in Figure K. Each column provided sharp symmetrical peaks, but note the elution pattern for the C18:3, C20:0, and C20:1 esters. From an SP-2330 column, these compounds elute in the order C20:0, C18:3, C20:1. As column polarity increases, retention of the C18:3 ester is increased. The C20:0 ester elutes first from an SP-2380 column, with the C18:3 and C20:1 esters coeluting. Elution order for the esters from an SP-2340 column – the most polar of the three columns – is C20:0, C20:1,

Figure K. Elution Order of FAMEs Changes with Increasing Column Polarity

Columns: **30m x 0.25mm ID, 0.20µm film**
Cat. No.: **24019 (SP-2330), 24110 (SP-2380), 24022 (SP-2340)**
Oven: 190°C
Carrier: helium, 20cm/sec
Det.: FID
Inj.: Rapeseed Oil Reference Mixture), split 100:1

1. C14:0
2. C16:0
3. C18:0
4. C18:1
5. C18:2
6. C22:0
7. C22:1



SP-2330 Column
Elution Order
C20:0
C18:3
C20:1

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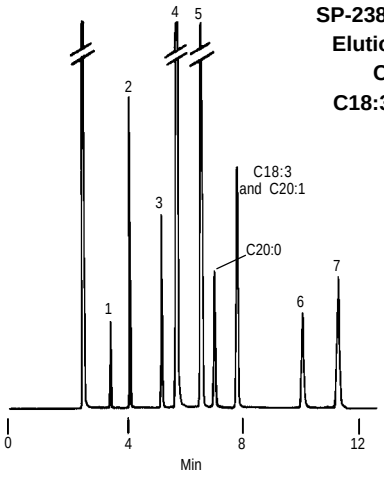

SP-2380 Column
Elution Order
C20:0
C18:3 - C20:1

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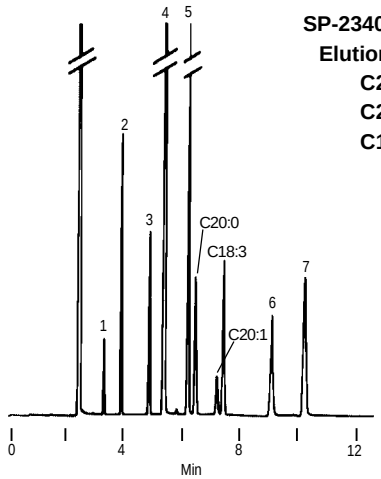


SP-2340 Column
Elution Order
C20:0
C20:1
C18:3

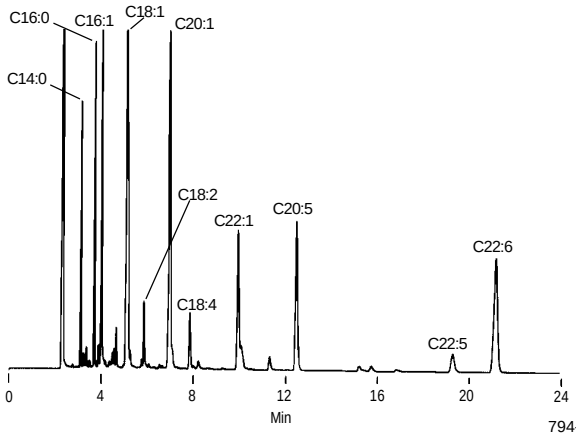
794-0495

C18:3. Note that coelution on the SP-2380 column occurs only under these conditions. It can be eliminated by changing the column temperature ±5°C.

The similarity of the chromatograms in Figure K indicates the SP-2380 column can be readily substituted for either of the other two columns. Because the differences in polarity are slight, only minor differences in elution order will be observed. Common compounds in Figures L and M, for example, elute in the same order. In addition to elution order differences among the cyanosilicone columns, these highly polar columns provide different elution characteristics from the same analysis performed on a less polar Omegawax or SUPELCOWAX 10 column. (Compare the patterns in Figure K to the pattern in Figure H, for example.) Thus, a cyanosilicone column can be useful when separation of specific FAMEs is unsatisfactory on an Omegawax or SUPELCOWAX 10 column.

Figure L. Polyunsaturated Fatty Acid Methyl Esters on an SP-2380 Column

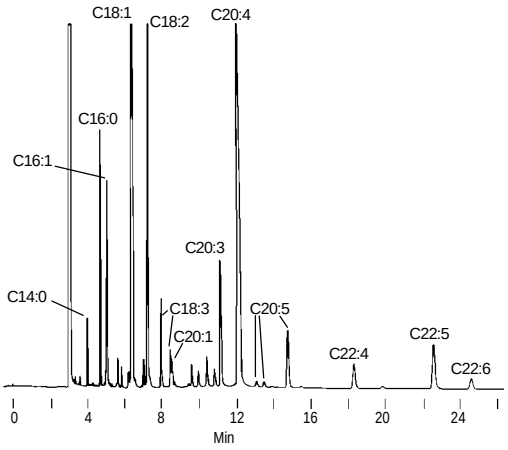
Column: **SP-2380, 30m x 0.25mm ID, 0.20µm film**
Cat. No.: **24110-U**
Oven: 190°C
Carrier: helium, 20cm/sec
Det.: FID
Inj.: PUFA No. 1 (Cat. No. 4-7033) in 0.2µL methylene chloride (5µg FAMEs on column), split 100:1



794-0496

Figure M. Polyunsaturated Fatty Acid Methyl Esters on an SP-2330 Column

Column: **SP-2330, 30m x 0.25mm ID, 0.20µm film**
Cat. No.: **24019**
Oven: 200°C
Carrier: helium, 20cm/sec set at 200°C
Det.: FID, 250°C
Inj.: 0.5µL chloroform containing natural source fatty acids, split 100:1, 250°C



713-0979

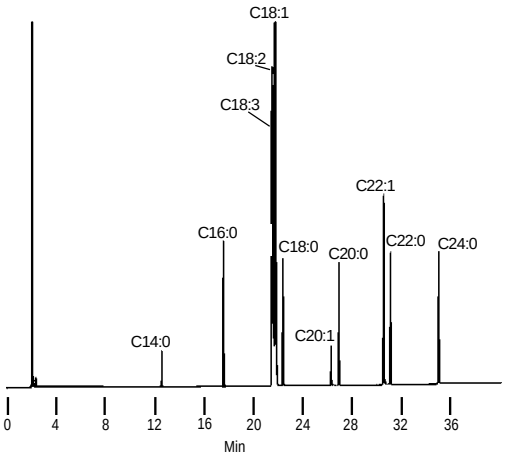
Nonpolar Columns

In analyses on nonpolar columns, fatty acid methyl esters are eluted by boiling point. For acids of each chain length, the unsaturated compounds elute before the saturated compound, which has the highest boiling point. This order is the reverse of the elution pattern from moderately polar and polar phase columns, and in some cases can offer an advantage. Unfortunately, however, there is considerable overlap in elution order among unsaturated acids of the same chain length. C18:2 is only partially resolved from C18:1, and C18:2 and C18:3 coelute, for example. The corresponding C20 and C22 acids elute in a similar, overlapping pattern (18). An example of the problem can be seen in the elution order of the C18 acid methyl esters in Figure N.

Because some of the C18-C22 acids are of nutritional importance (e.g., C18:2, linoleic acid, and C18:3, linolenic acid), such coelutions and overlaps make nonpolar columns unappealing to analysts monitoring food products.

Figure N. FAMEs on a Nonpolar Capillary Column

Column: **SPB-1, 30m x 0.20mm ID, 0.20µm film**
Cat. No.: **24163**
Oven: 150°C (4 min) to 280°C at 4°C/min, hold 4 min
Carrier: helium, 25cm/sec
Det.: FID
Inj.: 1.4µL of Rapeseed Oil Reference Mixture diluted to 50µg/µL, split 150:1



794-0497

Analyzing Positional *cis/trans* Isomers as Methyl Esters

Partially hydrogenated oils contain geometric and positional isomers of unsaturated fatty acids. Although some capillary columns are capable of separating groups of *cis* fatty acid methyl ester isomers from groups of *trans* isomers (e.g., SP-2330, SP-2380, and SP-2340 columns), it is difficult to further resolve components of either group. Some analysts who must resolve these complex mixtures must test two or three columns before finding one that effectively separates groups of *trans* isomers. Specially prepared 100-meter SP-2560 columns resolve positional *cis* or *trans* isomer groups well, with excellent column-to-column reproducibility.

SP-2560 columns are valuable for analyzing many complex FAME samples. Marine and terrestrial animals oils contain mono- and polyunsaturated fatty acids in the *cis* isomeric form. Natural forms of vegetable oils generally contain only *cis* isomers, but some of the double bonds are hydrogenated to provide a specific texture, or are converted to the *trans* form to increase thermal stability. An SP-2560 column enables you to study the composition of these fatty acid-containing materials. Similarly, you can monitor hydrogenation processes or determine the effects of different catalysts on hydrogenation by analyzing the isomer content of the product. Once you establish a process, you can use an SP-2560 column to routinely monitor *cis/trans* positional isomers in the product, and thus ensure that the process is functioning properly.

In addition to the proper column, other critical parameters in analyses of *cis/trans* isomer complexes are oven temperature, carrier gas linear velocity, and sample concentration (19). Note, for example, the change in the elution pattern for the C18:1 isomers when the temperature is decreased from 175°C (Figure O) to 170°C (Figure P). When the oven temperature is increased, *cis* isomers are better resolved from *trans* isomers in a complex mixture. Decreasing the oven temperature improves resolution of the *trans* isomers.

To determine the optimum temperature for a particular analysis, analyze a series of samples at temperature increments of 2°C (starting at 160°C) until optimum resolution is obtained. Because the temperature in a GC oven can differ by 5°C, or more, from the temperature set on the temperature controller, the oven temperature must be selected and monitored with care for the best overall separation of an isomer mixture.

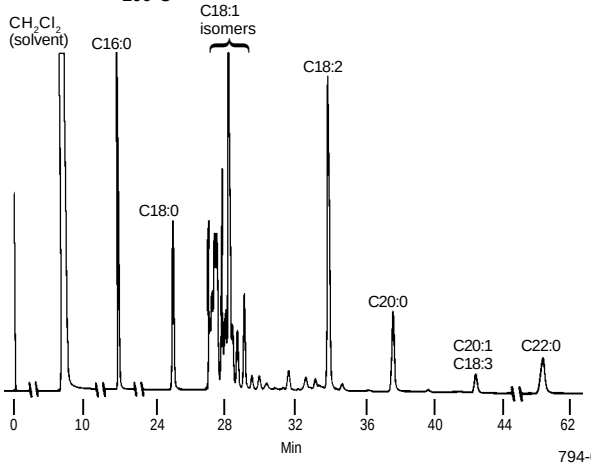
Similarly, for the best separations of positional *cis/trans* isomers, carrier gas flow must be set at the optimum average linear velocity ($\bar{\mu}$opt) for the gas used. If μ for helium is increased from 20cm/second ($\bar{\mu}$opt) to 30cm/second, resolution is lost (although analysis time for C18:1 isomers is reduced from 28 minutes to 14 minutes). Since nitrogen and hydrogen have different optimum linear velocities, each carrier gas may affect sample resolution differently. To duplicate the separations in Figures O and P, we recommend using helium at a $\bar{\mu}$ of 20cm/second as the carrier gas.

Sample concentration also affects the resolution of positional *cis/trans* isomers. Generally, better separations are obtained when the individual concentrations of closely eluting *trans* or *cis* isomers are less than 0.5mg/mL. (A 1µL injection of a 0.5mg/mL sample, split 100:1, provides 5ng of material on column.) Since it is impossible to control the concentration of each isomer, you should adjust the overall concentration of the mixture until the *trans* isomer complexes exhibit on-scale response at a detector sensitivity of 1×10^{-11} AFS or less.

At present no column will resolve all *cis/trans* isomers, but we test each SP-2560 column to ensure column-to-column reproducibility and maximum separation of most C18:1 positional *cis/trans* isomers (Figure O). Some 100-meter cyanosilicone columns will separate many of the same isomers, but these columns are not ordinarily tested for this purpose. Consequently, in analyses of complex mixtures of *cis/trans* isomers, two such columns often provide different results. You can use an SP-2330, SP-2380, SP-2340, or other cyanosilicone phase column when screening a sample for *cis* or *trans* isomer groups. But for consistent, maximum resolution of these isomers, use an SP-2560 column.

Figure O. Positional *cis/trans* Isomers

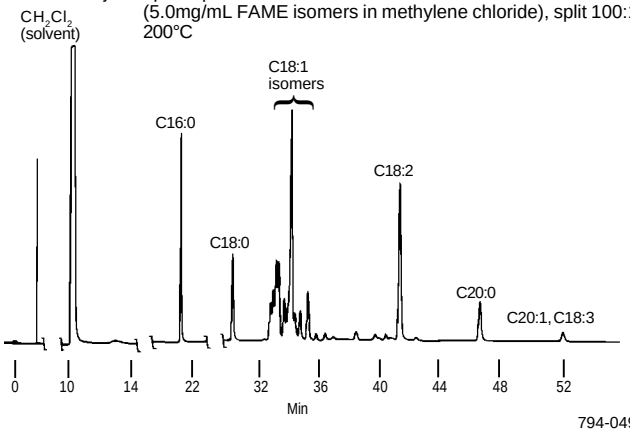
Column: **SP-2560, 100m x 0.25mm ID, 0.20µm film**
Cat. No.: **24056**
Oven: 175°C
Carrier: helium, 20cm/sec set at 175°C
Det.: FID, 200°C
Inj.: 1µL of positional *cis/trans* standard (5.0mg/mL FAME isomers in methylene chloride), split 100:1, 200°C



794-0498

Figure P. Decreasing Oven Temperature Changes *cis/trans* Isomer Resolution

Column: **SP-2560, 100m x 0.25mm ID, 0.20µm film**
Cat. No.: **24056**
Oven: 170°C
Carrier: helium, 20cm/sec set at 170°C
Det.: FID, 200°C
Inj.: 1µL of positional *cis/trans* standard (5.0mg/mL FAME isomers in methylene chloride), split 100:1, 200°C



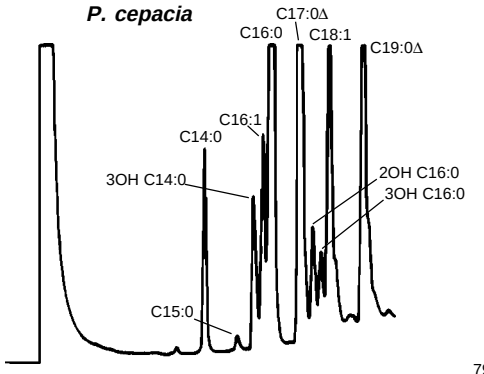
794-0499

Identifying Bacteria from Cellular C10-C20 Fatty Acid Profiles

Every species of anaerobic bacteria produces its own characteristic profile of cellular long chain fatty acids. Bacteriologists and others have compared gas chromatographic patterns of these compounds as a simple, reliable way of identifying even closely related species (Figure Q). Nonpolar capillary columns, such as SPB-1, resolve methyl esters of these fatty acids very well (Figure R), and a large library of information is based on the long-term use of nonpolar columns for this purpose. For more details about identifying bacteria through GC analyses, request **Application Note 394050**.

Figure Q. Closely Related Species of Bacteria Distinguished by Fatty Acid Profiles

Packing: **3% SP-2100 DOH on 100/120 SUPELCOPORT™**
Cat. No.: **12101** (20g)
Column: 10' x 2mm ID glass
Inj.: 1µL methyl esters of fatty acids from saponified whole cells of *Pseudomonas cepacia* or *P. aeruginosa*



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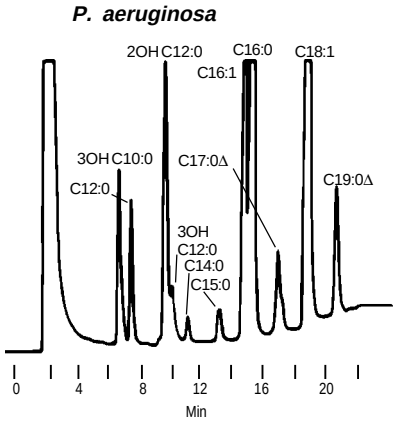
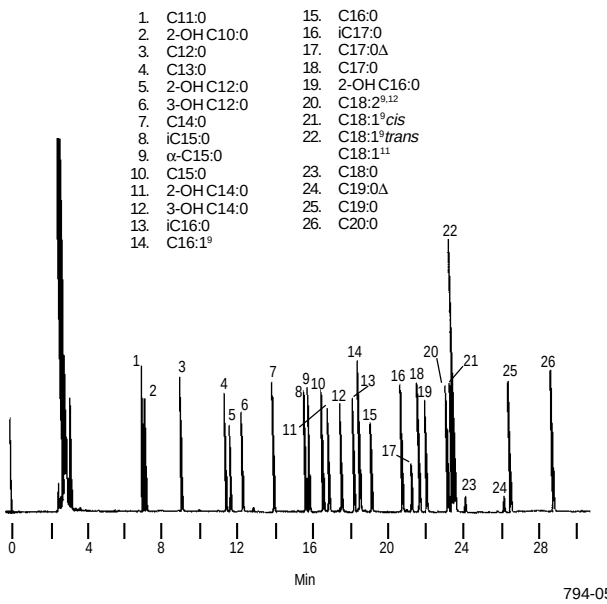


794-0501

Figure R. Bacterial Acid Methyl Esters

Column: **SPB-1, 30m x 0.25mm ID, 0.25µm film**
Cat. No.: **24028**
Oven: 150°C (4 min) to 250°C at 4°C/min
Carrier: helium, 20cm/sec
Det.: FID
Inj.: 1µL of Cat. No. 47080-U (10µg total FAMEs), split 100:1



794-0502

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References not available from Supelco.

Ordering Information:

Nukol Bonded Phase Fused Silica Columns

15m x 0.25mm ID, 0.25µm film	**24106-U**
30m x 0.25mm ID, 0.25µm film	**24107**
60m x 0.25mm ID, 0.25µm film	**24108**
15m x 0.32mm ID, 0.25µm film	**24130**
30m x 0.32mm ID, 0.25µm film	**24131**
60m x 0.32mm ID, 0.25µm film	**24132**
15m x 0.53mm ID, 0.50µm film	**25326**
30m x 0.53mm ID, 0.50µm film	**25327**

SP-2380 Stabilized Phase Fused Silica Columns

15m x 0.25mm ID, 0.20µm film	**24109**
30m x 0.25mm ID, 0.20µm film	**24110-U**
60m x 0.25mm ID, 0.20µm film	**24111**
15m x 0.32mm ID, 0.20µm film	**24115**
30m x 0.32mm ID, 0.20µm film	**24116**
60m x 0.32mm ID, 0.20µm film	**24117**
15m x 0.53mm ID, 0.20µm film	**25318**
30m x 0.53mm ID, 0.20µm film	**25319**

SP-2330 Nonbonded Phase Columns

Fused Silica

15m x 0.25mm ID, 0.20µm film	**24018**
30m x 0.25mm ID, 0.20µm film	**24019**
60m x 0.25mm ID, 0.20µm film	**24020-U**
15m x 0.32mm ID, 0.2µm film	**24102**
30m x 0.32mm ID, 0.20µm film	**24073**
60m x 0.32mm ID, 0.20µm film	**24074**

SP-2340 Nonbonded Phase Columns

Fused Silica

15m x 0.25mm ID, 0.20µm film	**24021**
30m x 0.25mm ID, 0.20µm film	**24022**
60m x 0.25mm ID, 0.20µm film	**24023**
15m x 0.32mm ID, 0.20µm film	**24138**
30m x 0.32mm ID, 0.20µm film	**24075**
60m x 0.32mm ID, 0.20µm film	**24076**

Omegawax 320 Bonded Phase Fused Silica Column

30m x 0.32mm ID, 0.25µm film	**24152**

Omegawax 250 Bonded Phase Fused Silica Column

30m x 0.25mm ID, 0.25µm film	**24136**

SUPELCOWAX 10 Bonded Phase Columns

Fused Silica

15m x 0.20mm ID, 0.20µm film	**24168**
30m x 0.20mm ID, 0.20µm film	**24169**
60m x 0.20mm ID, 0.20µm film	**24170**
15m x 0.25mm ID, 0.25µm film	**24077**
30m x 0.25mm ID, 0.25µm film	**24079**
60m x 0.25mm ID, 0.25µm film	**24081**
15m x 0.32mm ID, 0.25µm film	**24078**
30m x 0.32mm ID, 0.25µm film	**24080-U**
60m x 0.32mm ID, 0.25µm film	**24082**
15m x 0.32mm ID, 0.50µm film	**24083**
30m x 0.32mm ID, 0.50µm film	**24084**
60m x 0.32mm ID, 0.50µm film	**24085-U**
15m x 0.53mm ID, 0.50m film	**25324**
30m x 0.53mm ID, 0.50µm film	**25325**
15m x 0.53mm ID, 1.0µm film	**25300-U**
30m x 0.53mm ID, 1.0µm film	**25301**

Other dimensions are available; please refer to our current catalog, or inquire.

For example separations of polyunsaturated FAMEs on our SPB-PUFA column, request Application Note 395080.

SP-2560 Nonbonded Phase Fused Silica Column

100m x 0.25mm ID, 0.20µm film	**24056**

SPB-1 Bonded Phase Columns

Fused Silica

15m x 0.20mm ID, 0.20µm film	**24162**
30m x 0.20mm ID, 0.20µm film	**24163**
60m x 0.20mm ID, 0.20µm film	**24164**
15m x 0.25mm ID, 0.25µm film	**24026**
30m x 0.25mm ID, 0.25µm film	**24028**
60m x 0.25mm ID, 0.25µm film	**24030-U**
15m x 0.25mm ID, 1.0µm film	**24027**
30m x 0.25mm ID, 1.0µm film	**24029**
60m x 0.25mm ID, 1.0µm film	**24031**
15m x 0.32mm ID, 0.25µm film	**24099**
30m x 0.32mm ID, 0.25µm film	**24044**
60m x 0.32mm ID, 0.25µm film	**24046**
30m x 0.32mm ID, 1.0µm film	**24045-U**
60m x 0.32mm ID, 1.0µm film	**24047**
15m x 0.53mm ID, 0.50µm film	**25314**
30m x 0.53mm ID, 0.50µm film	**25315**
15m x 0.53mm ID, 1.5µm film	**25302-U**
30m x 0.53mm ID, 1.5µm film	**25303**

Column Protection

At elevated temperatures, polar capillary column phases – including cyanosilicone phases – are degraded by oxygen. To protect your column from trace amounts of oxygen in the carrier gas, use a high performance carrier gas purifier and regulator with a stainless steel diaphragm, and change the septum regularly. When you install the column, or after you change the septum, purge the column with purified carrier gas for at least 30 minutes before you heat it. For more information on carrier gas purification, request **Bulletins 848 and 918**.

Contact our Technical Service Department
(phone 800-359-3041 or 814-359-3041,
FAX 800-359-3044 or 814-359-5468)
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