Application Note

Food Testing

Agilent

Trusted Answers

# Improving the Analysis of 37 Fatty Acid Methyl Esters

## Using three types of capillary GC columns


**Authors**

Yun Zou
Agilent Technologies (Shanghai) Co.Ltd, Shanghai 200131 P.R.China

Hua Wu
Agilent Technologies (China) Co.Ltd, Beijing 100102 P.R.China



## Abstract

The analysis of fatty acid methyl esters (FAMEs) is used for the characterization of the lipid fraction in foods, and is one of the most important applications in food analysis. This application note details the separation of a 37-component FAME standard mixture on Agilent J&W CP-Sil 88 for FAME, Agilent J&W DB-FastFAME, and Agilent J&W DB-FATWAX Ultra Inert GC columns. Good resolution was demonstrated using the CP-Sil 88 for FAME GC column. The high-efficiency DB-FastFAME column provided excellent separation of the 37 FAMEs in only 8 minutes, while the DB-FATWAX Ultra Inert column offers unique selectivity for most saturated and polyunsaturated FAMEs.

## Introduction

Fats play an important role in the food nutrition and food chemistry areas of study. The fatty acid composition of fat is a complex mixture of saturated, monounsaturated, and polyunsaturated compounds with various carbon chain lengths. As the roles of fatty acids in the body vary depending on their structure, it is necessary to conduct detailed compositional analysis of the fatty acids contained in foods. The GC analysis of fatty acids as their methyl esters derivatives (FAMEs) is an important tool in the characterization of fats in the determination of total fat and *trans*-fat content in foods.[1,2] The choice of different stationary phases and other column dimensions such as column length, internal diameter, and film thickness depends mainly on the complexity of the fatty acid composition and the requirements in separation detail.

Routinely, polyethylene glycol (PEG) type capillary columns are used for FAME analysis of marine fish oils and meat samples, including the determination of butyric acid in milk fat. This is because PEG capillary columns elute the FAME isomers according to carbon chain length and degree of unsaturation. However, it has been reported that one of the more serious limitations of PEG columns is the lack of *cis-trans* differentiation. All *cis-trans* isomers coelute.[3]

Many regulatory methods for food testing require separation of specific *cis-trans* fatty acid isomers when determining fatty acid composition by GC/FID. For the analysis of more complex samples, such as edible oils, extra resolution of FAMEs is obtained using a capillary column coated with a cyanopropyl stationary phase. An Agilent J&W DB-FastFAME GC column with mid-content cyanopropyl phase provides fast and excellent separation for complex FAME mixtures and achieves some *cis/trans* separation. For more detailed *cis-trans* separation, the highly polar cyano-polysiloxane type column (CP-Sil 88 for FAME/HP-88) is preferred. However, some of the carbon chain lengths usually overlap on cyano-polysiloxane phases, causing problems in peak identification. Therefore, long GC columns (for example, 100 m) and long analysis times are required to achieve good FAME separations, however, this leads to low productivity.

The 37-component FAME standard mix is designed to mimic the fatty acid composition of many food samples, and it can be used to identify key fatty acid esters (FAMEs) in many foods. This mix contains FAMEs ranging from C4:0 to C24:1, including most of the important saturated, monounsaturated, and polyunsaturated FAMEs (Table 1).

This application note introduces the analysis of a 37-component FAME mix using three types of capillary columns designated for FAME analysis, including the Agilent J&W CP-Sil 88 for FAME, DB-FastFAME, and the Agilent J&W DB-FATWAX Ultra Inert GC column.

## Experimental

### Chemicals and standards

The 37-component FAME standard mixture (p/n CDAA-252795-MIX-1 mL) was purchased from ANPEL Scientific Instrument Co. Ltd (Shanghai, China). Table 1 lists the concentration of each component in the mixture.

PUFA No.1 (marine source), PUFA No.2 (animal source), and PUFA No.3 (from menhaden oil) were purchased from Minn Bolin Bio-Tech Co. LTD (Shenzhen, China). The mixture is available as 100 mg of neat mixture, which was diluted 100 times with acetone, respectively.

### Instrumentation

The analyses were performed using an Agilent 7890B GC equipped with a flame ionization detector (FID). Sample introduction was done using an Agilent 7683B automatic liquid sampler with a 5 µL syringe (p/n G4513-80213) and a split/splitless injection port. The instrumental configuration and analytical conditions are summarized in Table 2 (CP-Sil 88 for FAME column), Table 3 (DB-FastFAME column), Table 4 (high efficiency DB-FastFAME column), and Table 5 (DB-FATWAX UI column). Table 6 lists the other supplies used in this study.

**Table 1.** 37-component FAME mix.

| Number | Component (methyl esters) | Abbreviation | Concentration (mg/L) |
|---|---|---|---|
| 1 | Butyric acid | C4:0 | 403 |
| 2 | Caproic acid | C6:0 | 404 |
| 3 | Caprylic acid | C8:0 | 406 |
| 4 | Capric acid | C10:0 | 403 |
| 5 | Undecanoic acid | C11:0 | 200 |
| 6 | Lauric acid | C12:0 | 399 |
| 7 | Tridecanoic acid | C13:0 | 200 |
| 8 | Myristic acid | C14:0 | 397 |
| 9 | Myristoleic acid | C14:1 | 202 |
| 10 | Pentadecanoic acid | C15:0 | 202 |
| 11 | *cis*-10-Pentadecenoic acid | C15:1 | 200 |
| 12 | Palmitic acid | C16:0 | 599 |
| 13 | Palmitoleic acid | C16:1 | 200 |
| 14 | Heptadecanoic acid | C17:0 | 201 |
| 15 | *cis*-10-Heptadecenoic acid | C17:1 | 200 |
| 16 | Stearic acid | C18:0 | 399 |
| 17 | Oleic acid | C18:1 cis (n9) | 400 |
| 18 | Elaidic acid | C18:1 trans (n9) | 200 |
| 19 | Linoleic acid | C18:2 cis (n6) | 203 |
| 20 | Linolelaidic acid | C18:2 trans (n6) | 200 |
| 21 | γ-Linolenic acid | C18:3n6 | 203 |
| 22 | α-Linolenic acid | C18:3n3 | 199 |
| 23 | Arachidic acid | C20:0 | 406 |
| 24 | *cis*-11-Eicosenoic acid | C20:1(n9) | 199 |
| 25 | *cis*-11,14-Eicosadienoic acid | C20:2 | 200 |
| 26 | *cis*-8,11,14-Eicosatrienoic acid | C20:3n6 | 202 |
| 27 | *cis*-11,14,17-Eicosatrienoic acid | C20:3n3 | 200 |
| 28 | Arachidonic acid | C20:4n6 (ARA) | 198 |
| 29 | *cis*-5,8,11,14,17-Eicosapentaenoic | C20:5n3 (EPA) | 201 |
| 30 | Henicosanoic acid | C21:0 | 201 |
| 31 | Behenic acid | C22:0 | 400 |
| 32 | Erucic acid | C22:1n9 | 202 |
| 33 | *cis*-13,16-Docosadienoic acid | C22:2 | 199 |
| 34 | *cis*-4,7,10,13,16,19-Docosahexaenoic acid | C22:6(n3) (DHA) | 197 |
| 35 | Tricosanoic acid | C23:0 | 200 |
| 36 | Lignoceric acid | C24:0 | 405 |
| 37 | Nervonic acid | C24:1 | 201 |

**Table 2.** Agilent J&W CP-Sil 88 for FAME method conditions.

| Parameter | Value |
|---|---|
| GC system | Agilent 7890B/FID |
| Column | Agilent J&W CP-Sil 88 for FAME, 100 m × 0.25 mm, 0.20 µm (p/n CP7489) |
| Carrier gas | Helium, 32 psi, constant pressure mode |
| Inlet | Split/splitless, 260 °C, split ratio 50:1 |
| Oven | 100 °C (5 minutes),<br>8°C/min to 180 °C (9 minutes),<br>1 °C/min to 230 °C (15 minutes) |
| FID | 260 °C, Hydrogen: 40 mL/min<br>Air: 400 mL/min<br>Make-up gas: 25 mL/min |
| Injection | 1 µL |

Table 3. Agilent J&W DB-FastFAME method conditions.

| Parameter | Value |
|---|---|
| GC system | Agilent 7890B/ FID |
| Column | Agilent J&W DB-FastFAME, 30 m × 0.25 mm, 0.25 µm (p/n G3903-63011) |
| Carrier gas | Helium, 19 psi, constant pressure mode |
| Inlet | Split/splitless, 250 °C, split ratio 50:1 |
| Oven | 50 °C (0.5 minutes),<br>30 °C/min to 194 °C (3.5 minutes),<br>5 °C/min to 240 °C (1 minute) |
| FID | 280 °C, Hydrogen: 40 mL/min<br>Air: 400 mL/min<br>Make-up gas: 25 mL/min |
| Injection | 1 µL |

Table 4. High-efficiency Agilent J&W DB-FastFAME method conditions.

| Parameter | Value |
|---|---|
| GC system | Agilent 7890B/ FID |
| Column | Agilent J&W DB-FastFAME, 20 m × 0.18 mm, 0.20 µm (G3903-63010) |
| Carrier gas | Hydrogen, 28 psi, constant pressure mode |
| Inlet | Split/splitless, 250 °C, split ratio 50:1 |
| Oven | 80 °C (0.5 minutes),<br>65 °C/min to 175 °C,<br>10 °C/min to 185 °C (0.5 minutes),<br>7 °C/min to 230 °C |
| FID | 280 °C, Hydrogen: 40 mL/min<br>Air: 400 mL/min<br>Make-up gas: 25 mL/min |
| Injection | 1 µL |

Table 5. Agilent J&W DB-FATWAX Ultra Inert method conditions.

| Parameter | Value |
|---|---|
| GC system | Agilent 7890B/ FID |
| Column | Agilent J&W DB-FATWAX Ultra Inert, 30 m × 0.25 mm, 0.25 µm (p/n G3903-63008) |
| Carrier gas | Helium, constant flow mode. 30 cm/s |
| Inlet | Split/splitless, 250 °C, split ratio 50:1 |
| Oven | 40 °C (2 minutes),<br>55 °C/min to 171 °C (25 minutes),<br>10 °C/min to 215 °C (25 minutes) |
| FID | 280 °C, Hydrogen: 40 mL/min<br>Air: 400 mL/min<br>Make-up gas: 25 mL/min |
| Injection | 1 µL |

## Results and discussion

The highly polar cyanopropyl siloxane phases, such as CP-SIL 88 for FAME or HP-88, were designed with the express purpose of improving separations of *cis-trans* FAMEs. The efficacy of the high-polarity cyanopropyl siloxane columns for *trans* fatty acid determinations has been successfully demonstrated previously. However, there is significant carbon chain overlap in the elution patterns for 37-component FAMEs analysis in many applications, for example, C18:3n6 or C18:3n3, and C20:0; C20:3n3, C22:1n9, and C20:4n6.[3] This can lead to peak identification problems. Figure 1 shows the optimized method separation of the 37-component FAMEs reference standard using a CP-Sil 88 for FAME column and GC-FID, resulting in excellent selectivity; all 37 components were baseline resolved in one run.

To achieve good resolution for all compounds in 37-component FAMEs standard mixture, a 100 m CP-Sil 88 for FAME GC column was selected, and the analysis time was more than 70 minutes.

Table 6. Flowpath supplies.

| Parameter | Value |
|---|---|
| Vials | Amber, write-on spot, certified, 2 mL, screw top vial packs (p/n 5182-0554) |
| Septa | Nonstick BTO septa (p/n 5183-4757) |
| Column nut | Self tightening, inlet/detector (p/n 5190-6194) |
| Ferrules | 15% Graphite: 85% Vespel, short, 0.4 mm id, for 0.1 to 0.25 mm columns (10/pk, p/n 5181-3323) |
| Liner | Agilent Ultra Inert split liner with glass wool (p/n 5190-2295) |
| Inlet seal | Ultra Inert, gold-plated, with washer (p/n 5190-6144) |

Another common alternative that is used for analysis of these types of FAMEs in complex mixtures is the mid-content cyanopropyl phase GC columns. The J&W DB-FastFAME GC column was specifically engineered for the fast separation of FAME mixtures. Due to the stronger interaction of *cis* isomers with the cyano-dipole, the *trans* isomers elute before the *cis* isomers. Therefore, they can provide some separation of *cis* and *trans* FAMEs.

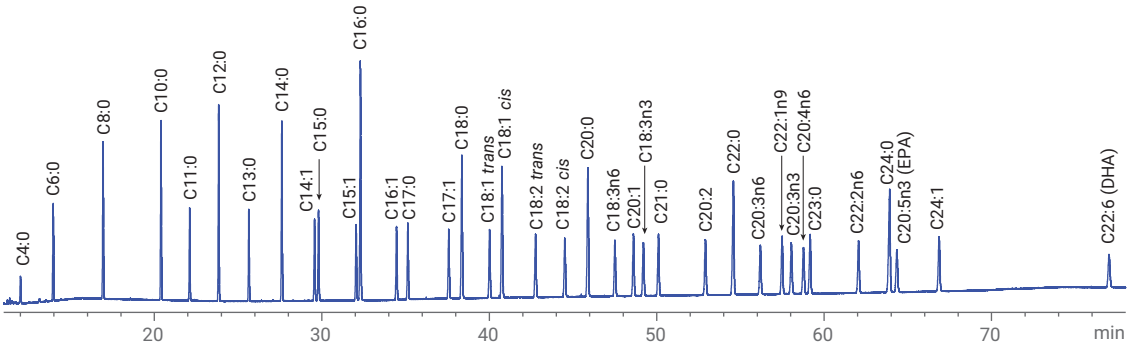



**Figure 1.** GC/FID chromatogram of 37-component FAMEs standard mixture on a 100 m × 0.25 mm id, 0.25 µm Agilent J&W CP-Sil 88 for FAME column using Method 1 (See Table 2).

## The J&W DB-FastFAME GC column

A 30 m × 0.25 mm id, 0.25 µm DB-FastFAME column was selected to analyze the 37-component FAME standard mixture. Figure 2 shows the typical GC-FID chromatogram. All compounds in the standard mixture were well resolved, and the analysis time was less than 18 minutes.

A high-efficiency 0.18 mm id GC column is one possible way to improve productivity without losing measurement performance. This is because decreasing the internal diameter results in an increase of the column efficiency per meter; the column length can be reduced while keeping the resolution constant. The use of hydrogen as a carrier gas provides a faster analysis with almost equivalent resolution because the optimum linear carrier gas velocity is higher due to the higher diffusivity of hydrogen. Figure 3 shows the separation of the 37-component FAME standard mixture on the 20 m × 0.18 mm id, 0.20 µm DB-FastFAME column. The method completely resolved all compounds (resolution > 1.5) in the standard mix including AOAC critical pairs, and reduced run times to under 8 minutes, indicating the possibility that fast sample throughput can be achieved using the high-efficiency columns without compromising resolution.

Different elution order of two pairs of compounds: EPA/c22:0 and DHA/C24:1 in Figures 2 and 3 can be observed. Changing inlet pressure in a temperature program run may change the effective temperatures the compounds experience. The method for analysis of EPA and DHA in complex mixtures can be optimized by changing elution order of these two pairs of compounds on DB-FastFAME GC column if changing carrier gas, using different inlet pressure and film thickness.

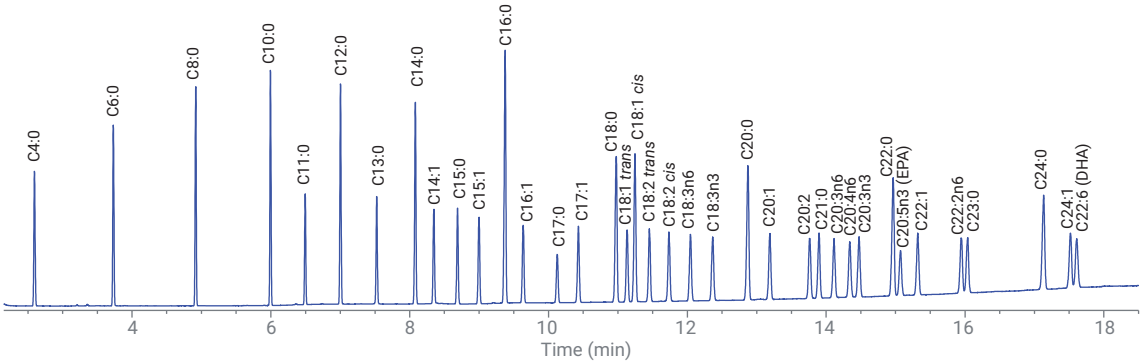



**Figure 2.** GC/FID chromatogram of 37-component FAMEs standard mixture on a 30 m × 0.25 mm id, 0.25 µm Agilent J&W DB-FastFAME column using Method 2 (see Table 3).

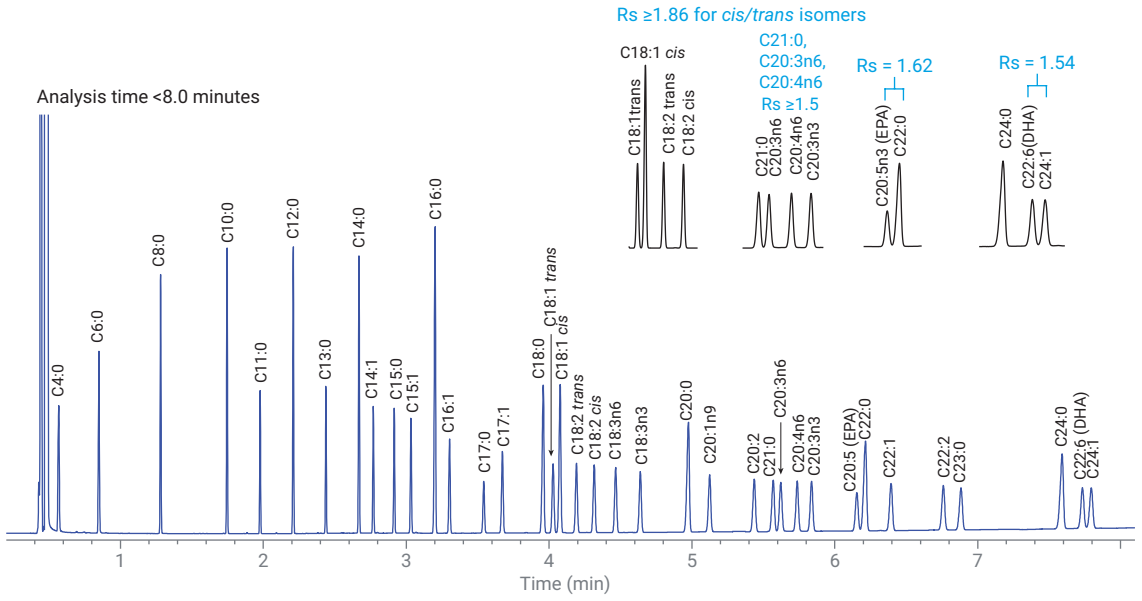



**Figure 3.** GC/FID chromatogram of 37-component FAMEs standard mixture on a 20 m × 0.18 mm id, 0.20 µm Agilent J&W DB-FastFAME column using Method 3 (see Table 4).

Routinely, PEG columns (WAX columns) are useful for FAME analysis of classical edible oils and fats, marine fish oils, and meat samples, including the determination of butyric acid in milk fat. This is because they will elute the FAME isomers according to carbon chain length and degree of unsaturation. But there is some overlap, for example, C20:3 n6 and C21:1 fail to separate, and C22:6 and C24:1 also coelute; no separation of *cis-trans* isomers was obtained on a traditional WAX column.

The J&W DB-FATWAX Ultra Inert (UI) GC column was introduced with improved performance. Figure 4 shows the separation of the 37-component FAME standard mixture on the 30 m × 0.25 mm id, 0.25 µm DB-FATWAX Ultra Inert GC column. A good separation is obtained, except for one pair of C18:1 *cis* and C18:1 *trans* isomers; resolution is 0.56. This pair of compounds normally coelutes, appearing as one peak on other WAX columns. Another pair of *cis-trans* isomers, C18:2 *cis* and C18:2 *trans*, can be baseline separated on a DB-FATWAX UI GC column, indicating that DB-FATWAX UI columns offer some resolution of *cis* and *trans* isomers, with the *cis* isomer eluting first.

A group of polyunsaturated fatty acids (PUFAs) having multiple double bonds of importance for human nutrition are the Omega-3 and Omega-6 fatty acids, such as C20:5n3 (EPA), C22:6n3 (DHA), and C20:4n6 (ARA). Figure 4 shows good resolution for these Omega-3 and Omega-6 FAMEs. Figures 5 through 7 give examples of the separation obtained for mixtures of PUFAs. Key FAMEs including EPA and DHA can easily be detected and quantified.

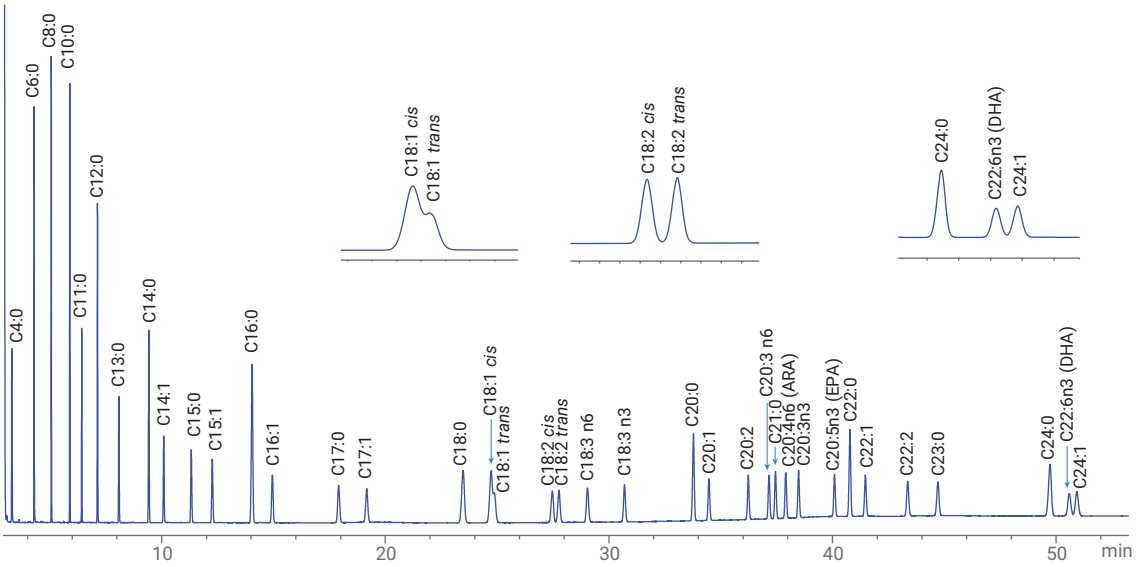
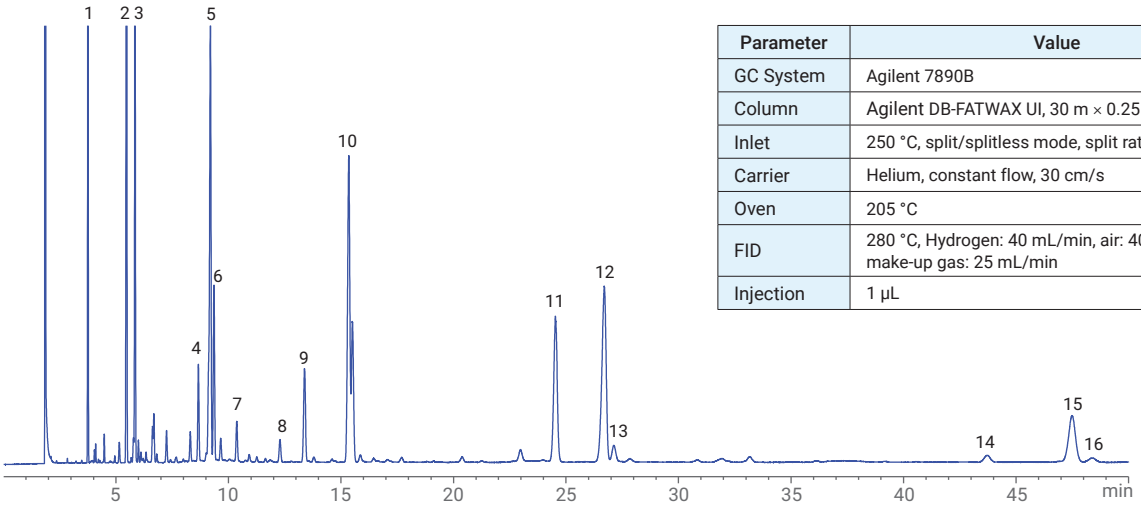


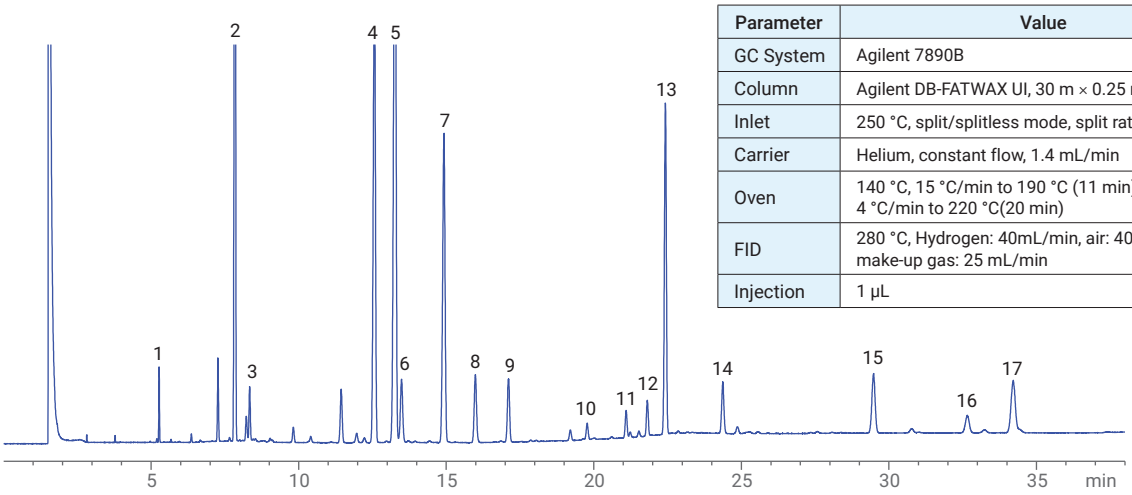

**Figure 4.** GC/FID chromatogram of 37-component FAMEs standard mixture on a 30 m × 0.25 mm id, 0.25 µm Agilent J&W DB-FATWAX Ultra Inert GC column using Method 4 (see Table 5).




| Parameter | Value |
|---|---|
| GC System | Agilent 7890B |
| Column | Agilent DB-FATWAX UI, 30 m × 0.25 mm, 0.25 µm |
| Inlet | 250 °C, split/splitless mode, split ratio 100:1 |
| Carrier | Helium, constant flow, 30 cm/s |
| Oven | 205 °C |
| FID | 280 °C, Hydrogen: 40 mL/min, air: 400 mL/min, make-up gas: 25 mL/min |
| Injection | 1 µL |

1. C14:0
2. C16:0
3. C16:1n7
4. C18:0
5. C18:1n9
6. C18:1n7
7. C18:2n6
8. C18:3n3
9. C18:4n3
10. C20:1n9
11. C20:5n3 (EPA)
12. C22:1n11
13. C22:1n9
14. C22:5n3
15. C22:6n3 (DHA)
16. C24:1n9

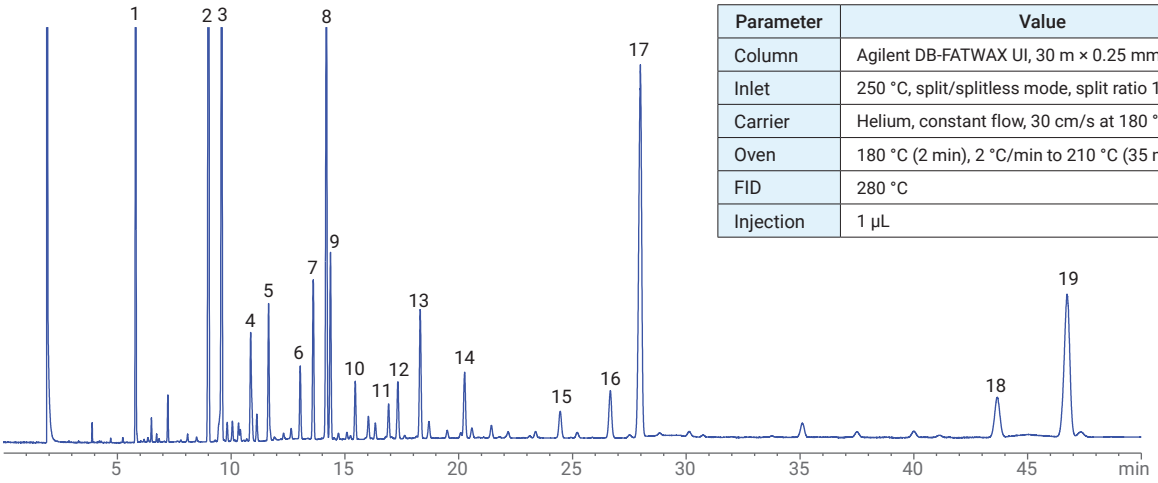
**Figure 5.** GC/FID chromatogram of PUFA No.1 mix (marine source) on a 30 m × 0.25 mm id, 0.25 µm Agilent J&W DB-FATWAX Ultra Inert GC column .




| Parameter | Value |
|---|---|
| GC System | Agilent 7890B |
| Column | Agilent DB-FATWAX UI, 30 m × 0.25 mm, 0.25 µm |
| Inlet | 250 °C, split/splitless mode, split ratio 100:1 |
| Carrier | Helium, constant flow, 1.4 mL/min |
| Oven | 140 °C, 15 °C/min to 190 °C (11 min), 4 °C/min to 220 °C(20 min) |
| FID | 280 °C, Hydrogen: 40mL/min, air: 400 mL/min, make-up gas: 25 mL/min |
| Injection | 1 µL |

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

**Figure 6.** GC/FID chromatogram of PUFA No.2 mix (animal source) on a 30 m × 0.25 mm id, 0.25 µm Agilent J&W DB-FATWAX Ultra Inert GC column.




| Parameter | Value |
|---|---|
| Column | Agilent DB-FATWAX UI, 30 m × 0.25 mm, 0.25 µm |
| Inlet | 250 °C, split/splitless mode, split ratio 100:1 |
| Carrier | Helium, constant flow, 30 cm/s at 180 °C |
| Oven | 180 °C (2 min), 2 °C/min to 210 °C (35 min) |
| FID | 280 °C |
| Injection | 1 µL |

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. C18:3n3
12. C18:3n4
13. C18:4n3
14. C20:1n9
15. C20:4n6 (ARA)
16. C20:4n3
17. C20:5n3(EPA)
18. C22:5n3
19. C22:6n3(DHA)

**Figure 7.** GC/FID chromatogram of PUFA No.3 mix (from menhaden oil) on a 30 m × 0.25 mm id, 0.25 µm Agilent J&W DB-FATWAX Ultra Inert GC column.

## Conclusions

This application note introduces an improved analysis of 37-component FAME standard mix using three types of capillary columns designated for FAME analysis. The highly polar cyanopropyl siloxane phase Agilent J&W CP-Sil 88 for FAME column was the preferred column choice for the separation of *cis*- and *trans*-isomers. Baseline separation of all components in 37-component FAME standard mix on CP-Sil 88 for FAME was demonstrated in this work. An Agilent J&W DB-FastFAME GC column can also provide excellent resolution for analysis of 37-component FAME standard mix. A high-efficiency 0.18 mm id DB-FastFAME GC column can completely resolve all compounds in the standard mix, and reduce run times to under 8 minutes. It provides the possibility of achieving fast sample throughput using high-efficiency columns without compromising resolution. The Agilent J&W DB-FATWAX UI column offers unique selectivity for most saturated and polyunsaturated FAMEs. Except for C18:1 *cis-trans* isomers, other FAMEs including C18:2 *cis* and *trans* isomers, such as ARA, EPA, and DHA can be well separated. The column is ideal for analysis of fish oil, meat fat, and key Omega 3 and Omega 6 FAMEs.

## References


1. AOAC Official Methods of Analysis (**2000**), method Ce 2–66.
2. IUPAC, Standard methods for Analysis of Oils, Fats and Derivatives, Blackwell Scientific Publications, IUPAC Method 2.301.
3. F. David, P. Sandra, A. K. Vickers. Column selection for the Analysis of Fatty Acid Methyl Esters. *Agilent Technologies Application Note*, publication number 5989-3760EN, **2005**.



**www.agilent.com**

DE65829273





Printed in the USA, October 30, 2023
5991-8706EN


Agilent
Trusted Answers