Application Note

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Rapid Analysis of 37 FAMEs with the Agilent 8860 Gas Chromatograph

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Abstract

An Agilent 8860 GC equipped with a split/splitless inlet and flame ionization detector (FID) was used to analyze a 37-component fatty acid methy ester (FAME) standard mixture and real samples of proteolytic formula. This Application Note describes analysis of a mixture of 37 FAMEs that is faster than GB 5009.168-2016, with excellent separation.

Introduction

Fatty acids are the main components of neutral fats, phospholipids, and glycolipids. Fatty acids can be divided into three categories: saturated, monounsaturated, and polyunsaturated. Some fatty acids, such as linoleic acid, are essential fatty acids; they cannot be synthesized by the body, and must be obtained from food. Omega-3 and omega-6 fatty acids, such as arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), are polyunsaturated fatty acids that play a key role in human nutrition. Omega-3 fatty acids are believed to be involved in preventing diseases such as heart disease and high blood pressure. Fats play a very important role in nutrition and food chemistry.

Hydrolysis and methylation are the most commonly used methods for fatty acid determination; esterification of fatty acids reduces polarity, and facilitates the separation of unsaturated isomers. The analysis of fatty acid methyl esters (FAMEs) is one of the most important applications in food analysis.

GB 5009.168-2016 regulates the determination of 37 fatty acids in food. Sample pretreatment and gas chromatography (GC) parameters are described in detail in this method, which recommends a highly polar cyano-polysiloxane-type column (100 m × 0.25 mm, 0.2 µm). The method enables separation of 37 FAMEs in 82 minutes. A previous Agilent Application Note shows that the Agilent Intuvo 9000 GC system with an Agilent J&W DB-FastFAME Intuvo GC column, using helium as the carrier gas, can resolve 37 FAMEs within eight minutes[2]. This rapid analysis benefits from Intuvo direct heating technology, which allows the column to be heated at 250 °C/min.

This Application Note describes relatively rapid methods with two different lengths of DB-FastFAME columns using the 8860 GC system with nitrogen as the carrier gas. The RSDs of area were determined, and key neutral fats such as ARA, EPA, and DHA were quantitatively analyzed. To demonstrate the method applicability, infant formula samples were also analyzed in this application.

Experimental

Chemicals and standards

37-Component FAME standard mixture (p/n CDAA-252795-MIX-1 mL) and single standards of C20:4n6 (p/n CDAA-253207M-10 mg), C20:5n3 (p/n CDAA-253209M-10 mg), and C22:6n3 (p/n CDAA-253228M-10 mg) were purchased from ANPEL Scientific Instrument Co. Ltd. (Shanghai, China). The concentration of each component in the mixture was 200 to 400 mg/mL.

Samples of infant formula were obtained locally. Sample pretreatment was performed according to GB 5009.168-2016.

Instrumentation

FAMEs analyses were performed using an 8860 GC equipped with a flame ionization detector (FID). Sample introduction was done using an Agilent 7693A automatic liquid sampler with a 5 µL syringe and a split/splitless injection port. Tables 1 and 2 show the instruments and conditions.

Table 1. Agilent DB-FastFAME 30 m × 0.25 mm, 0.25 µm method conditions.

Parameter	Value
GC system	8860 GC/FID
Inlet	Split/splitless, 250 °C, split ratio 100:1; Liner (p/n 5190-2295)
Column	DB-FastFAME, 30 m × 0.25 mm, 0.25 µm (p/n G3903-63011)
Carrier	Nitrogen, 12 psi, constant pressure
Oven	80 °C (0.5 minutes), then 40 °C/min to 165 °C (1 minute), then 4 °C/min to 230 °C (4 minutes)
FID	260 °C; hydrogen: 40 mL/min; air: 400 mL/min; make-up gas (N_2): 25 mL/min
Injection	1 µL

Table 2. Agilent DB-FastFAME 20 m × 0.18 mm, 0.2 µm method conditions.

Parameter	Value
GC system	8860/FID
Inlet	Split/splitless, 250 °C, split ratio 100:1; Liner (p/n 5190-2295)
Column	DB-FastFAME, 20 m × 0.18 mm, 0.2 µm (p/n G3903-63010)
Carrier	Nitrogen, 20 psi, constant pressure
Oven	80 °C, then 35 °C/min to 194 °C (1 minute), then 5 °C/min to 245 °C
FID	260 °C; hydrogen: 40 mL/min; air: 400 mL/min; make-up gas (N_2): 25 mL/min
Injection	1 µL

Results and discussion

Figure 1 shows a typical chromatogram for the analysis of the 37 FAMEs standard mixture, obtained on the 30-m DB-FastFAME column. In some applications, *cis*- and *trans*-C18:1, *cis*- and *trans*-C18:2, C22:0, and C20:3, and C22:6 and C24:1 may coelute and cause peak identification problems. As Figure 1 demonstrates, using these conditions, all 37 compounds were well separated through the 8860 GC system, with sharp, symmetrical peaks and runtime reduced to under 24 minutes. Of particular note is the ability to separate the *cis-trans* isomers and the EPA and DHA components. This method is very helpful for the quantitative analysis of fatty acids in complex mixtures, especially for the determination of EPA and DHA in a matrices such as fish oil. Compared to a 0.18 mm id column, the larger id and thicker film of this 30 m × 0.25 mm, 0.25 µm column provides greater column capacity and longer column lifetime.

Proteolytic formula is a good choice for babies with milk allergies, and can be divided into three types according to the degree of hydrolysis: partial hydrolysis formula, deep hydrolysis formula, and amino acid formula. Figures 2 through 4 show the analysis of the three different types of proteolytic formula. Key FAMEs, including C18:2n6, C18:3n3, ARA, and DHA, were easily detected and quantified.

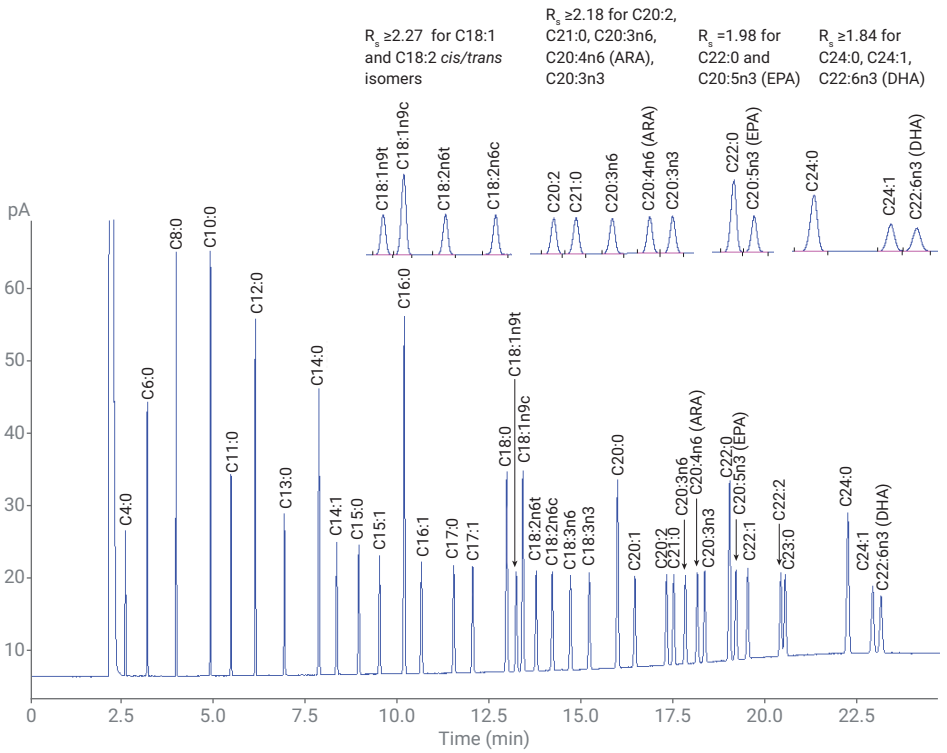

Figure 1. GC/FID chromatogram of 37 component FAMEs standard mixture on a 30 m × 0.25 mm, 0.25 µm DB-FastFAME column (p/n G3903-63011).

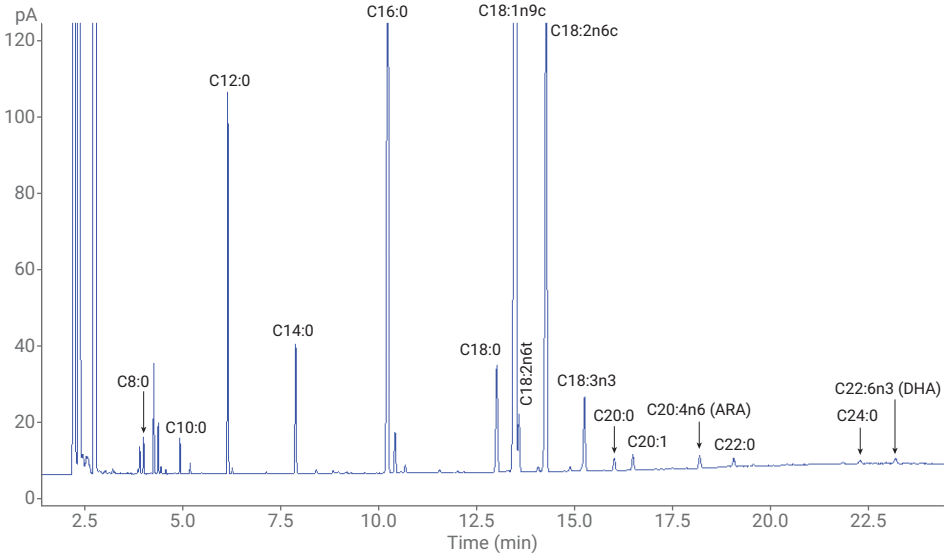

Figure 2. GC/FID chromatogram of FAMEs in amino acid formula on a 30 m × 0.25 mm, 0.25 µm DB-FastFAME column.

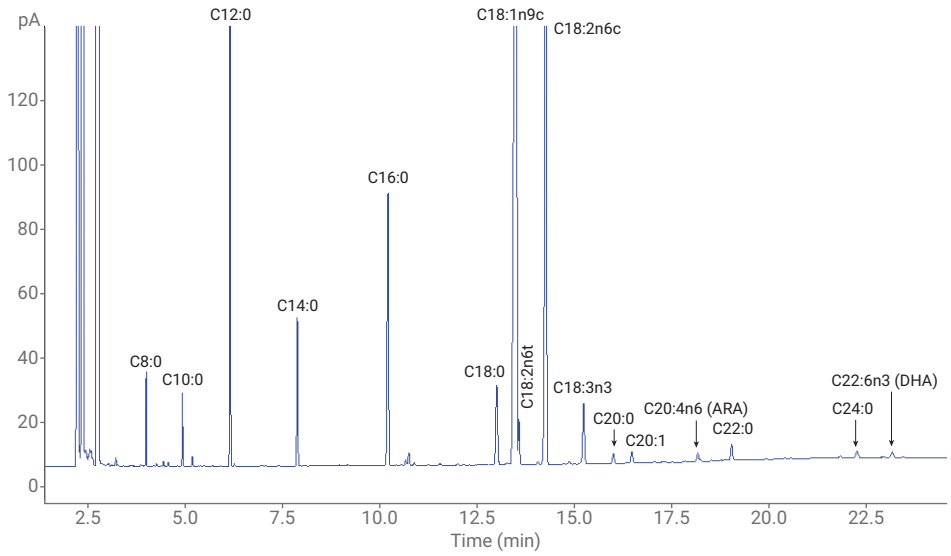

Figure 3. GC/FID chromatogram of FAMEs in deep hydrolysis formula on a 30 m × 0.25 mm, 0.25 µm DB-FastFAME column.

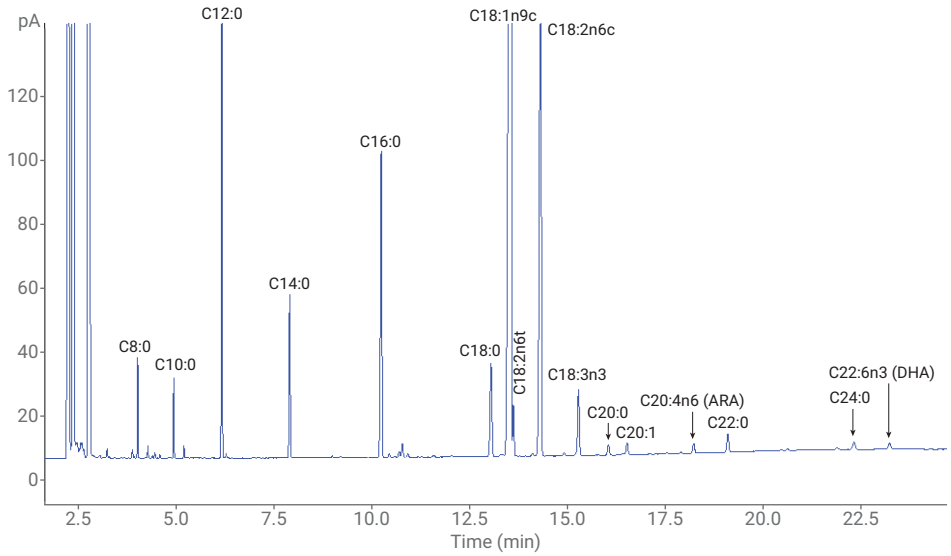

Figure 4. GC/FID chromatogram of FAMEs in partial hydrolysis formula on a 30 m × 0.25 mm, 0.25 µm DB-FastFAME column.

Calibration standards of ARA, EPA, and DHA were prepared at concentrations of 5, 10, 50, 100, 250, and 1,000 mg/L. The coefficient of determination (R^2) for those three compounds were ≥0.9997. Figures 5, 6, and 7 show the calibration curves.

Method repeatability was tested using six injections of the standard mixture. Figure 8 illustrates that for all compounds except C4:0, the area RSD% was well below 1 %. This demonstrates that the 8860 GC system is a reliable system for analyzing fatty acids.

A high-efficiency 0.18 mm id column has the potential to improve productivity, and reduce the run time without losing analytical performance. As demonstrated in Figure 9, the use of this short column provides faster analysis—under 14 minutes—with almost equivalent resolution. The resolution values of key compounds are shown in Figures 1 and 9.

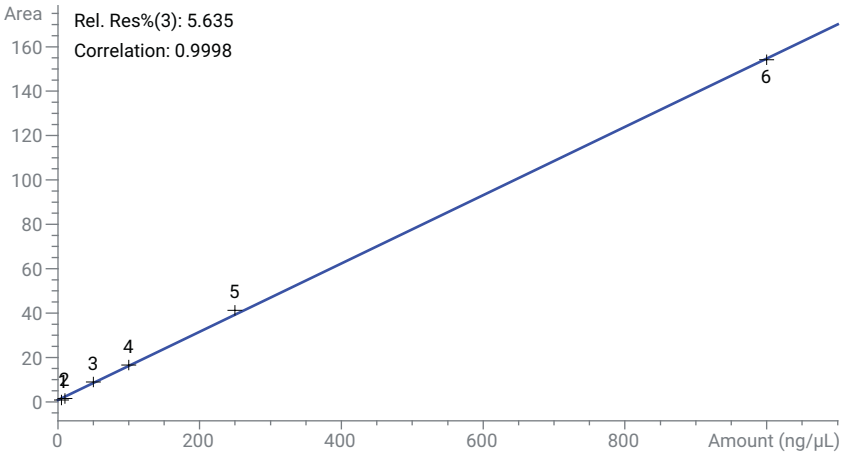

Figure 5. Calibration of C20:4n6 (ARA) from 5 to 1,000 µg/mL on a 30 m × 0.25 mm, 0.25 µm DB-FastFAME column.

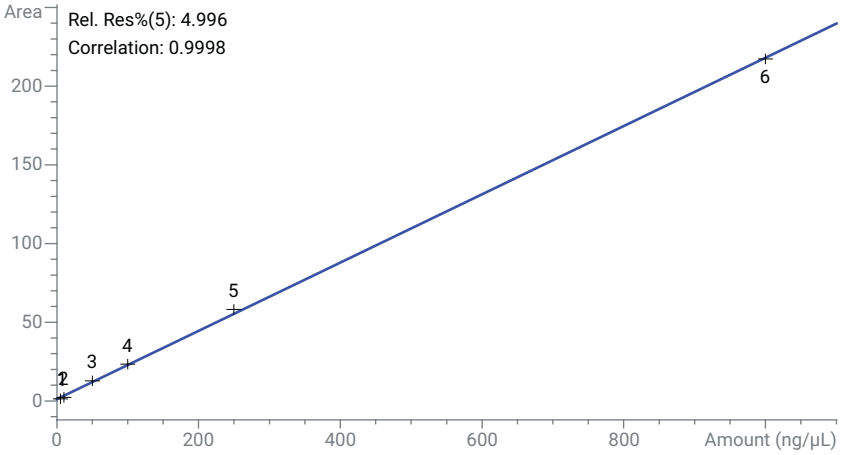

Figure 6. Calibration of C20:5n3 (EPA) from 5 to 1,000 µg/mL on a 30 m × 0.25 mm, 0.25 µm DB-FastFAME column.

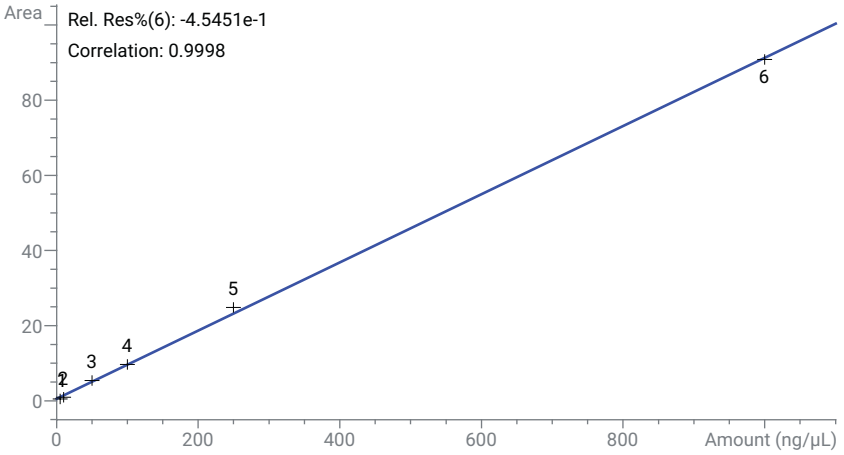

Figure 7. Calibration of C22:6n3 (DHA) from 5 to 1,000 µg/mL on a 30 m × 0.25 mm, 0.25 µm DB-FastFAME column.

Conclusion

This Application Note demonstrates that the 8860 GC configured with automatic injection and an FID can provide a faster, reliable solution for the analysis of 37 FAMEs. Two types of capillary column designated for FAMEs analysis were used; both show excellent efficiency without compromising resolution. The 30 m DB-FastFAME column provides greater column capacity and better durability, while the 20 m DB-FastFAME column provides shorter runtime. The 7693A autosampler, with a capacity of 16 sample vials, and 8860 GC EPC control ensure good repeatability and ease-of-operation suitable for the fast food industry as well as routine analysis.

References

1. Zhou, Y.; Wu, H. Improving the analysis of 37 fatty acid methyl esters, *Agilent Technologies Application Note*, publication number 5991-8706EN, **2018**.
2. Zhou, Y. Rapid separation of fatty acid methyl esters, *Agilent Technologies Application Note*, publication number 5994-0116EN, **2018**.

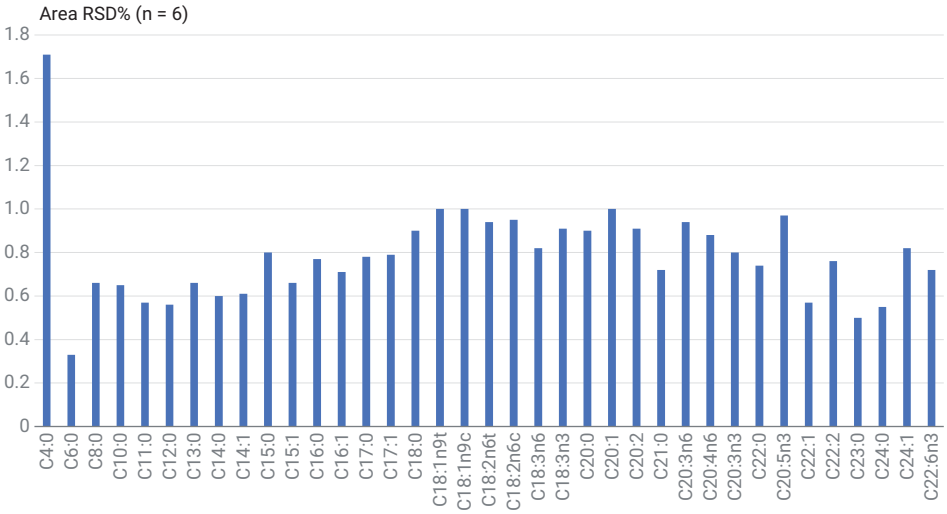

Figure 8. Area RSD% of six repeat injections with 37-component FAMEs standard mixture on a 30 m × 0.25 mm, 0.25 µm DB-FastFAME column.

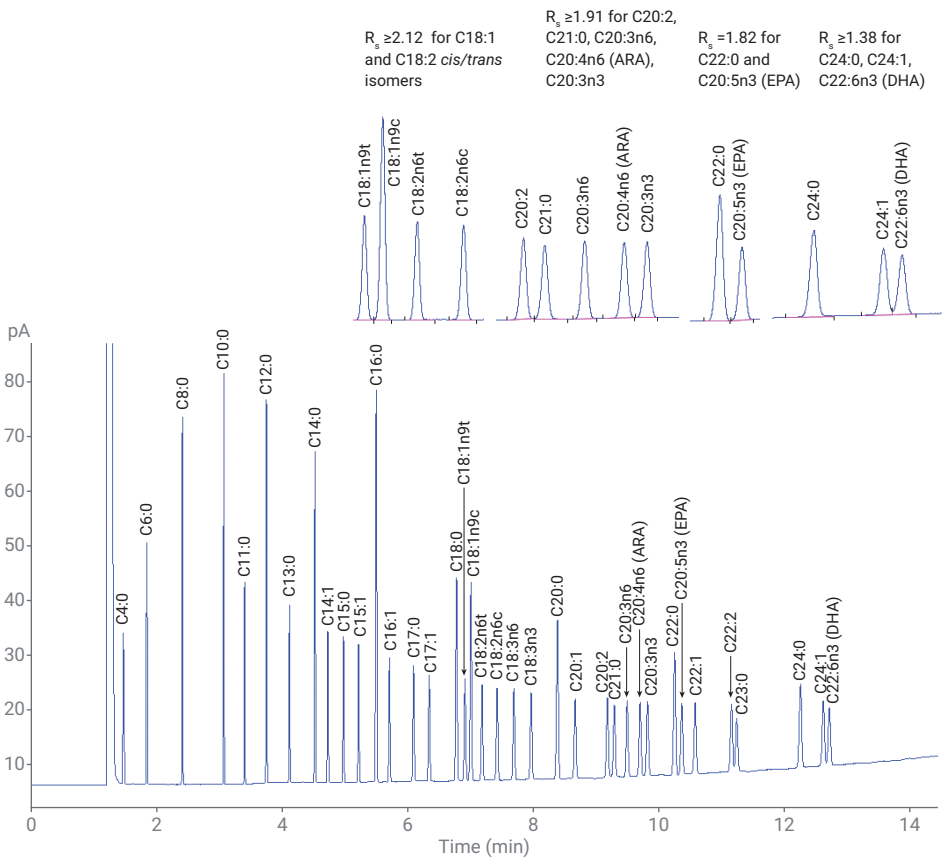

Figure 9. GC/FID chromatogram of 37 component FAMEs standard mixture on a 20 m × 0.18 mm, 0.2 µm DB-FastFAME column (p/n G3903-63010).

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