Application Note

Food Testing

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Rapid Analysis of Fatty Acid Methyl Esters (FAMEs) Using a High Resolution 90-m Agilent J&W DB-FastFAME Capillary GC Column

Author

Yun Zou
Agilent Technologies
(Shanghai) Co. Ltd.

Abstract

This application note shows an excellent separation of a 37-component and 63-component FAME standard mixture. The separation was performed on a 90 m Agilent J&W DB-FastFAME GC column using helium and hydrogen as carrier gasses. Compared with helium as a carrier gas, the analysis time is reduced by more than 25% by using hydrogen. The analysis time of a 37-component FAME mixture can be achieved in less than 25 minutes while maintaining excellent resolution for all compounds. A separation of a 63-component FAME mixture, including several C18:1, C18:2, and C18:3 *cis-trans* positional isomers, can be completed within 35 minutes.

Introduction

Many regulatory methods for food testing require separation of specific *cis-trans* fatty acid isomers when determining fatty acid composition by GC/FID. The highly polar biscyanopropyl or cyano-polysiloxane type columns are often used to provide the necessary resolution. Some of the carbon chain lengths can overlap on these cyano-polysiloxane phases, causing problems in peak identification. Therefore, long GC columns (i.e. 100 m) and long analysis times (i.e. more than 70 minutes) are required to achieve good FAME separations. The longer analysis times can lead to decreased productivity.

In this application note, a new high-resolution cyanopropyl phase, DB-FastFAME, engineered for the fast separation of FAMEs, is evaluated. The 90 m DB-FastFAME, along with an optimized temperature program, and hydrogen as carrier gas can effectively resolve critical FAMEs with better resolution and faster analysis times than traditional 100 m cyanopropyl GC columns.

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).

One Chinese customer provided the 63-component FAME standard mixture. The FAME standards mix (GLC-569-B) was purchased by this customer from Nu-Chek Prep, Inc. (Elysian, Mn). Linoleic Acid Methyl Ester mix (*cis/trans*, CRM CRM47791) and Linolenic Acid Methyl Ester isomer mix (CRM47792) were obtained from Sigma-Aldrich (China). The rest of individual FAME standards were obtained from ANPEL Scientific Instrument Co. Ltd (Shanghai, China).

Each FAME was dissolved in *n*-hexane to a final concentration of approximately 0.015 to 0.15 mg/mL.

Instrumentation

The analyses were performed using an Agilent 8890 GC equipped with a flame ionization detector (FID). Sample introduction was done using an Agilent G4513A automatic liquid sampler with 5 µL syringe (p/n G4513-80213), and a split/splitless injection port. The instrumental configuration and analytical conditions are summarized in Tables 1 to 4. Other supplies used in this study are listed in Table 5.

Table 1. 37-component FAME mix method 1.

GC System	Agilent 8890/FID
Column	Agilent J&W DB-FastFAME, 90 m × 0.25 mm, 0.25 µm (p/n G3903-63013, s/n: US0123221H)
Carrier Gas	Helium, 58 psi, constant pressure mode
Inlet	Split/splitless, 260 °C, split ratio 50:1
Oven	80 °C (1 min), 40 °C/min to 200 °C (18 min), 8 °C/min to 235 °C (15 min)
FID	260 °C, hydrogen: 40 mL/min; air: 400 mL/min; make-up gas: 25 mL/min
Injection	1 µL

Table 2. 37-component FAME mix method 2.

GC System	Agilent 8890/FID
Column	Agilent J&W DB-FastFAME, 90 m × 0.25 mm, 0.25 µm (p/n G3903-63013, s/n: US0123221H)
Carrier Gas	Hydrogen, 40 psi, constant pressure mode
Inlet	Split/splitless, 260 °C, split ratio 50:1
Oven	80 °C (1 min), 40 °C/min to 200 °C (8 min), 4 °C/min to 235 °C (15 min)
FID	260 °C, hydrogen: 40 mL/min; air: 400 mL/min; make-up gas: 25 mL/min
Injection	1 µL

Table 3. 63-component FAME mix method 1.

GC System	Agilent 8890/FID
Column	Agilent J&W DB-FastFAME, 90 m × 0.25 mm, 0.25 µm (p/n G3903-63013, s/n: US0123221H)
Carrier Gas	Helium, 46 psi, constant pressure mode
Inlet	Split/splitless, 260 °C, split ratio 30:1
Oven	75 °C (1 min), 40 °C/min to 200 °C (20 min), 2 °C/min to 208 °C (1 min), 8 °C/min to 230 °C (20 min)
FID	260 °C, hydrogen: 40 mL/min; air: 400 mL/min; make-up gas: 25 mL/min
Injection	1 µL

Table 4. 63-component FAME mix method 2.

GC System	Agilent 8890/F ID
Column	Agilent J&W DB-FastFAME, 90 m x 0.25 mm, 0.25 µm (p/n G3903-63013, s/n: US0123221H)
Carrier Gas	Hydrogen, 30 psi, constant pressure mode
Inlet	Split/splitless, 260 °C, split ratio 30:1
Oven	75 °C (1 min), 40 °C/min to 200 °C (12 min), 2 °C/min to 208 °C (1 min), 6 °C/min to 230 °C (15 min)
FID	260 °C, hydrogen: 40 mL/min; air: 400 mL/min; make-up gas: 25 mL/min.
Injection	1 µL

Table 5. Flow path supplies

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)

Results and discussion

The 37-component FAME standard mix is designed to mimic the fatty acid composition of many food samples and can be used to identify key FAMEs in many foods. It takes approximately 70 minutes for a 37-fame mix analysis using helium as carrier gas and a 100 m high-content cyanopropyl column to obtain the necessary resolution of the key components. But Figure 1 shows the excellent separation of this typical mix in under 38 minutes on a 90 m DB-FastFAME GC column using helium carrier gas. All 37 components are baseline separated because of the good selectivity of the DB-FastFAME cyanopropyl phase.

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. Higher gas velocity is due to the higher diffusivity of hydrogen. The separation of the 37-component FAME standard mixture on the 90 m × 0.25 mm id, 0.25 µm DB-FastFAME column is shown in Figure 2. Like method 1, method 2 completely resolved all compounds (resolution >1.5) in the standard mix including AOAC critical pairs and reduced run times to under 25 minutes. This resolution indicates that fast sample throughput can be achieved with the high-resolution columns in routine work.

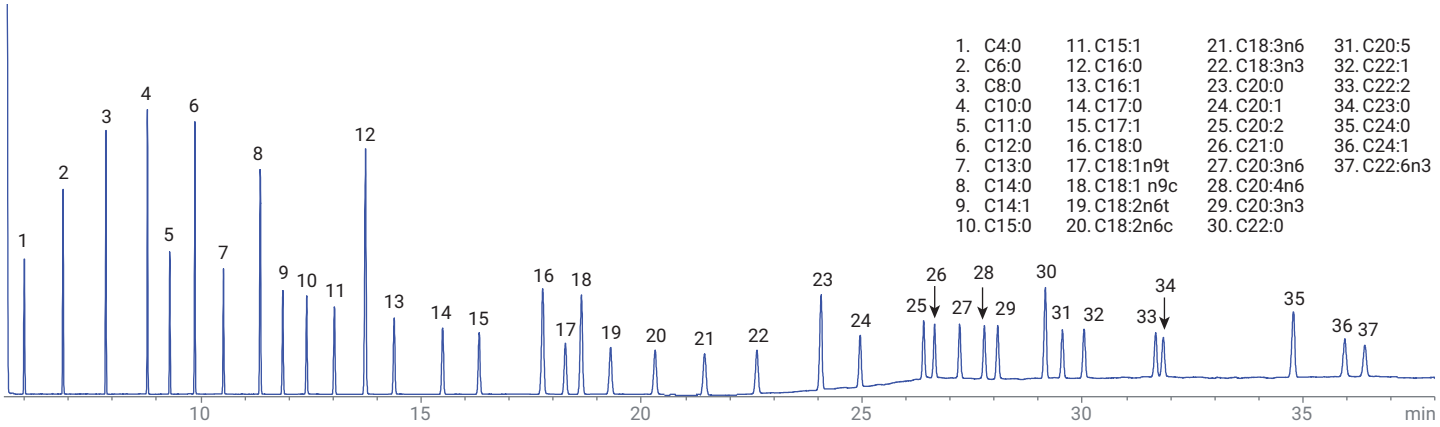

Figure 1. GC/FID chromatogram of 37-component FAMEs standard mixture on a 90 m × 0.25 mm id, 0.25 µm Agilent J&W DB-FastFAME column using helium as carrier gas (method see Table 1).

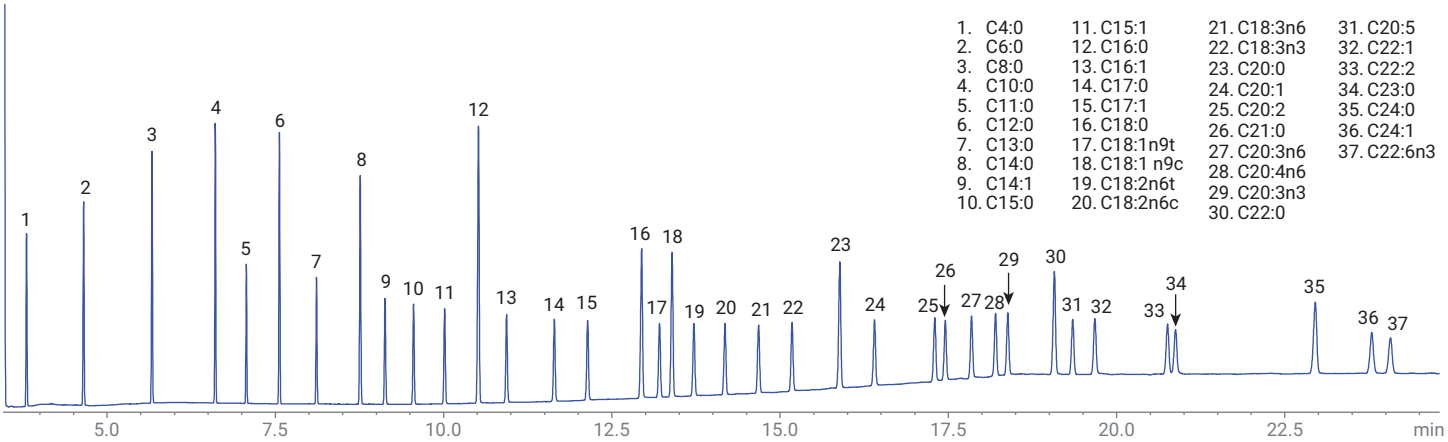

Figure 2. GC/FID chromatogram of 37-component FAMEs standard mixture on a 90 m × 0.25 mm id, 0.25 µm Agilent J&W DB-FastFAME column using hydrogen as carrier gas (method see Table 2).

The measurement of *trans* fatty acid content in processed foods for nutritional labeling is one of the most important applications in food analysis. Analysis of *cis-trans* FAMEs is very challenging; so far, no single column can separate all positional FAME isomers. GC with a 100 m high-content cyanopropyl column is widely used for detailed separation of *cis* and the corresponding *trans* isomers. However, analysis times are generally too long, and there is significant carbon chain overlap in the elution patterns for FAMEs analysis in many applications. For example, the peak of 11c-20:1 and that of 9t, 12c, 15c-18:3 overlapped partially or completely. The J&W DB-FastFAME GC column was engineered for the fast separation of FAME mixtures. The stronger interaction of *cis* isomers with the cyano-dipole causes the *trans* isomers to elute before the *cis* isomers. Therefore, they can provide some separation of *cis* and *trans* FAMEs.

The 63-component FAME standard mix includes some common *trans-cis* positional isomers, such as several C18:1, C18:2, and C18:3 *cis-trans* positional isomers. A 90 m × 0.25 mm id, 0.25 µm DB-FastFAME column was used to analyze the 63-component FAME standard mixture; the typical GC-FID chromatograms are shown in Figures 3A and 4A. The J&W DB-FastFAME GC column has different polarity and selectivity than the high-content cyanopropyl column. Therefore, the peak of 11c-20: 1 (peak 45) does not interfere with the determination of 9t, 12c, 15c-18:3 (peak 38). With helium carrier gas, the 90 m DB-FastFAME can effectively separate the 63-component FAME standard mix within 48 minutes. Challenging positional isomers

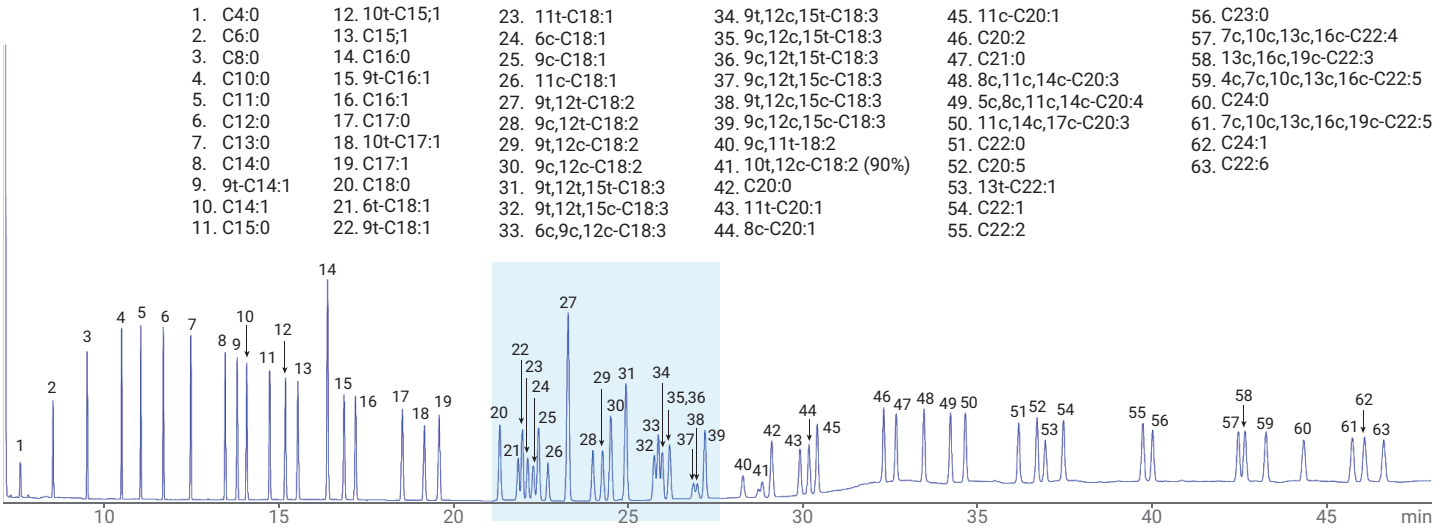

Figure 3A. GC/FID chromatogram of 63-component FAMEs standard mixture on a 90 m × 0.25 mm id, 0.25 µm Agilent J&W DB-FastFAME column using helium as carrier gas (method see Table 3).

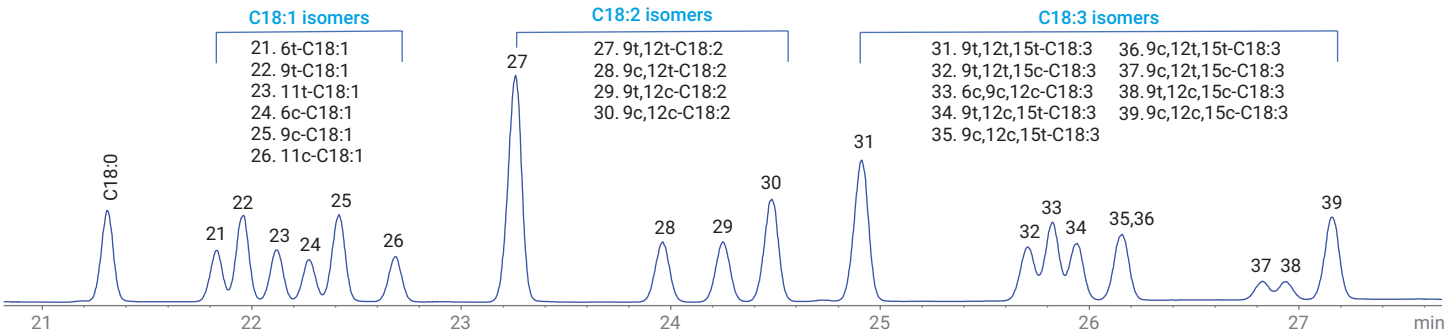

Figure 3B. Enlarged section of GC/FID chromatogram of 63-component FAMEs standard mixture shown in Figure 3A.

(see Figure 3B), including the 11t-C18:1 and 6c-C18:1 critical pair, can be well resolved (Rs = 1.4). All C18:2 isomers can be baseline separated. Only one pair of 9c, 12t, 15t-C18:3 and 9c, 12c, 15t-C18:3 coelute. But this pair of compounds normally coelutes as one peak on 100 m high-content cyanopropyl columns. Peak 33 (6c, 9c, 12c-C18:3) is not baseline separated from peak 32 (9t, 12t, 15c-C18:3) and peak 34 (9t, 12c, 15t-C18:3); resolution of two pairs of compounds is approximately 0.8. Lowering or increasing the flow rate or the corresponding oven temperature can move the peak 33 forward or backward to improve resolution if necessary. As shown in Figure 4A and 4B, hydrogen can give a faster separation than helium with all 63 components eluting in less than 35 minutes with similar and even better resolution.

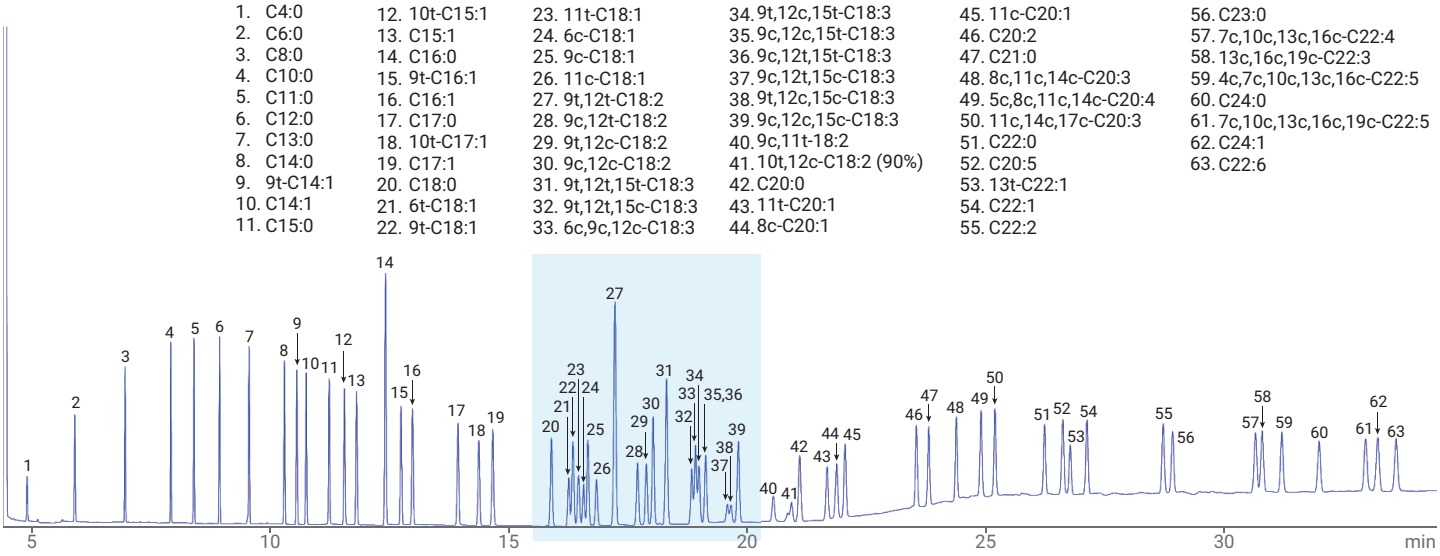

Figure 4A. GC/FID chromatogram of 63-component FAMEs standard mixture on a 90 m × 0.25 mm id, 0.25 µm Agilent J&W DB-FastFAME column using hydrogen as carrier gas (method see Table 4).

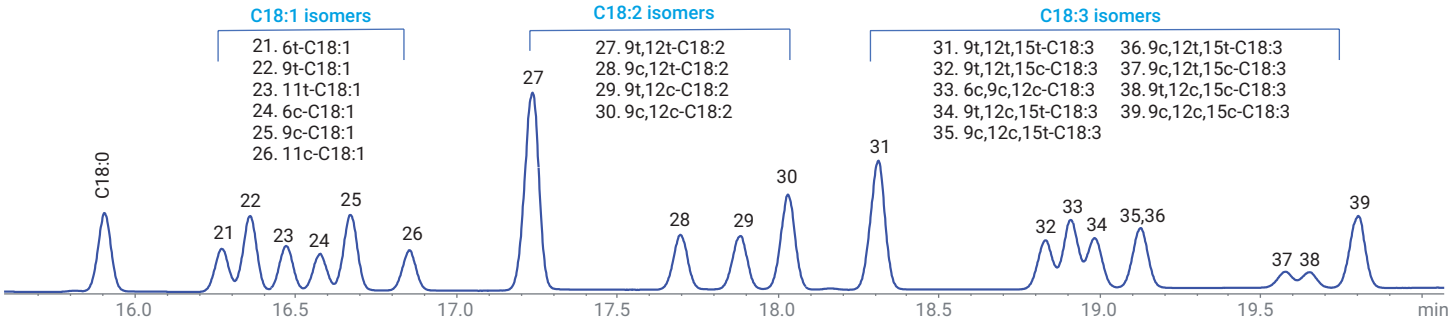

Figure 4B. Enlarged section of GC/FID chromatogram of 63-component FAMEs standard mixture shown in Figure 4A.

Conclusion

This application note introduces the analysis of a 37-component FAME standard mix and a 63-component FAME standard mix with a 90 m Agilent J&W DB-FastFAME capillary column using helium and hydrogen as carrier gas. Baseline separation of all components in the 37-component FAME standard mix within 38 minutes using helium carrier gas and less than 25 minutes using hydrogen carrier gas was demonstrated in this work. The 90 m high-resolution Agilent J&W DB-FastFAME can also effectively separate the 63-component FAME standard mix including common C18:1, C18:2, and C18:3 isomers within 48 minutes using helium carrier gas. The use of hydrogen carrier gas can reduce run times to under 35 minutes without compromising resolution.

The high-resolution Agilent J&W DB-FastFAME GC column can provide fast and excellent separation for complex FAME mixtures and achieves *cis-trans* isomer separation for routine analysis work.

References

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