Application Note

Food Testing & Agriculture

Rapid Separation of Fatty Acid Methyl Esters

Using DB-FastFAME Intuvo GC columns

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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 introduces rapid, good separation of FAME standard mixtures using an Agilent Intuvo 9000 GC system with an Agilent J&W DB-FastFAME Intuvo GC column.

Introduction

The analysis of oils, fat, and fat-containing food is a common task in governmental, quality control (QC), or contract research organization (CRO) laboratories. The GC analysis of fatty acids as their FAME derivatives is an important tool in the characterization of fats in the determination of total fat and *trans*-fat content in foods[1,2]. Many regulatory methods for testing foods such as edible oils require separation of specific *cis/trans* fatty acid isomers using a capillary column coated with a cyanopropyl stationary phase when determining fatty acid composition. In addition, long GC columns (100 m) and long analysis times (more than 70 minutes) are required to achieve good FAME separations[3,4]. However, this leads to high analysis costs and low productivity.

The DB-FastFAME Intuvo GC column with a cyanopropyl phase was specifically engineered for the fast separation of FAME mixtures, including some key cis/*trans* separations, to meet the requirements of regulatory methods. This Application Note briefly demonstrates rapid analysis of FAME standard mixtures using the DB-FastFAME Intuvo GC column and the Intuvo 9000 GC system.

Experimental

Chemicals and standards

The FAME 36 component mixture (p/n 5191-4276), C4–C24 even carbon saturated FAME mixture (p/n 5191-4278), and FAME mix, rapeseed oil (p/n 5191-4277) were from Agilent Technologies. The 37-component FAME standard mixture (p/n CDAA-252795-MIX-1 mL) was purchased from ANPEL Scientific Instrument Co. Ltd. (Shanghai, China).

The C4–C24 even carbon saturated FAME mixture was prepared by dilution in hexane to a concentration of 500 µg/mL. The FAME mixture of rapeseed oil is available as a 100 mg neat mixture, which was diluted 20 times with dichloromethane.

Instrumentation

The analyses were performed using an Intuvo 9000 GC equipped with a flame ionization detector (FID). Sample introduction was done using an Agilent 7693A automatic liquid sampler with 5 µL syringe (p/n G4513-80213), and a split/splitless injection port.

Table 1. Method 1 conditions.

GC system	Intuvo 9000 GC/FID
Column	J&W DB-FastFAME, 20 m × 0.18 mm, 0.20 µm, Intuvo module (p/n G3909-63005)
Carrier gas	Helium, 50 psi, constant pressure mode
Inlet	Split/splitless, 260 °C, split ratio 100:1
Oven	50 °C (0.3 minutes), 250 °C/min to 175 °C, 20 °C/min to 240 °C (2 minutes)
Guard Chip	200 °C
FID	260 °C, Hydrogen: 40 mL/min Air: 400 mL/min Make up gas: 25 mL/min
Injection	1 µL

Table 2. Method 2 conditions.

GC system	Intuvo 9000 GC/FID
Column	J&W DB-FastFAME, 20 m × 0.18 mm, 0.20 µm, Intuvo module (p/n G3909-63005)
Carrier gas	Hydrogen, 28 psi, constant pressure mode
Inlet	Split/splitless, 260 °C, split ratio 100:1
Oven	50 °C (0.3 minutes), 200 °C/min to 200 °C (0.4 minutes), 20 °C/min to 240 °C (1 minute)
Guard Chip	200 °C
FID	260 °C, Hydrogen: 40 mL/min Air: 400 mL/min Make up gas: 25 mL/min.
Injection	1 µL

Table 3. Method 3 conditions.

GC system	Intuvo 9000 GC/FID
Column	J&W DB-FastFAME, 20 m × 0.18 mm, 0.20 µm, Intuvo module (p/n G3909-63005)
Carrier gas	Helium, 32 psi, constant pressure mode
Inlet	Split/splitless, 260 °C, split ratio 100:1
Oven	50 °C (0.3 minutes), 250 °C/min to 175 °C, 10 °C/min to 190 °C (0.5 minutes), 13 °C/min to 240 °C (2 minutes)
Guard Chip	200 °C
FID	260 °C, Hydrogen: 40 mL/min Air: 400 mL/min Make up gas: 25 mL/min.
Injection	1 µL

Results and discussion

The FAME 36 component standard mix is designed to mimic the fatty acid composition of many food samples; it can be used to identify key 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. Not included in this mix is tricosanoic acid methyl ester (C23:0), a FAME that was previously used as an internal standard. Figure 1 shows the separation of the FAME 36 component mixture on the 20 m × 0.18 mm, 0.20 µm DB-FastFAME Intuvo GC column. The method with helium as the carrier gas completely resolves all compounds within five minutes, including AOAC critical pairs, with R_s >1.5.

Table 4. Method 4 conditions.

GC system	Intuvo 9000 GC/FID
Column	J&W DB-FastFAME, 20 m × 0.18 mm, 0.20 µm, Intuvo module (p/n G3909-63005)
Carrier gas	Hydrogen, 18 psi, constant pressure mode
Inlet	Split/splitless, 260 °C, split ratio 100:1
Oven	50 °C (0.3 minutes), 150 °C/min to 185 °C (0.5 minutes), 15 °C/min to 230 °C (5 minutes)
Guard Chip	200 °C
FID	260 °C, Hydrogen: 40 mL/min Air: 400 mL/min Make up gas: 25 mL/min
Injection	1 µL

Table 5. Flowpath supplies.

Vials	Amber, write-on spot, certified, 2 mL, screw top vial packs (p/n 5182–0554)
Septa	Inlet septa, nonstick, BTO (p/n 5183–4757)
Guard Chip	Guard Chip, Intuvo, split/splitless inlet (p/n G4587-60565)
Liner	Agilent Ultra Inert inlet liner, split, with glass wool (p/n 5190–2295)

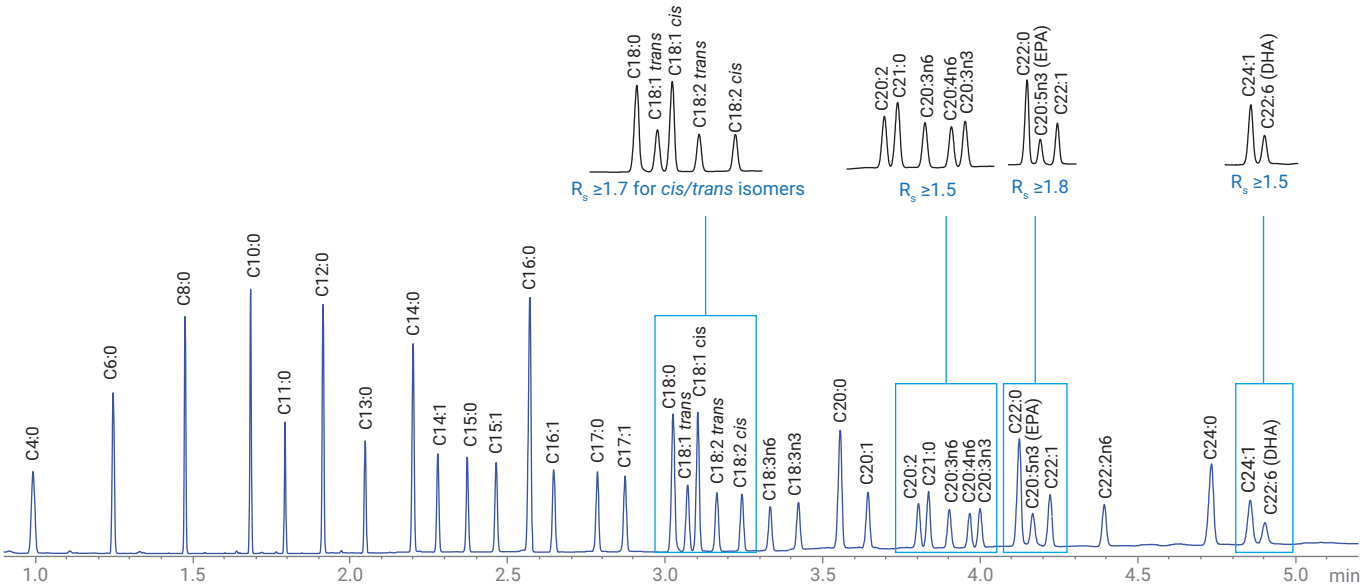

Figure 1. GC/FID chromatogram of the FAME 36 component mixture on a 20 m × 0.18 mm, 0.20 µm DB-FastFAME Intuvo column using Method 1 with helium as carrier gas (see Table 1).

Figure 2 shows the typical GC/FID chromatogram of the FAME mix with rapeseed oil. Using this method, good peak shape and resolution was obtained, with an analysis time of five minutes.

Using hydrogen as carrier gas, the C4–C24 even carbon saturated FAME mixture and the FAME 36 component mixture can be well separated in less than four minutes (Figures 3 and 4).

This indicates that fast sample throughput can be achieved with the column without compromising resolution.

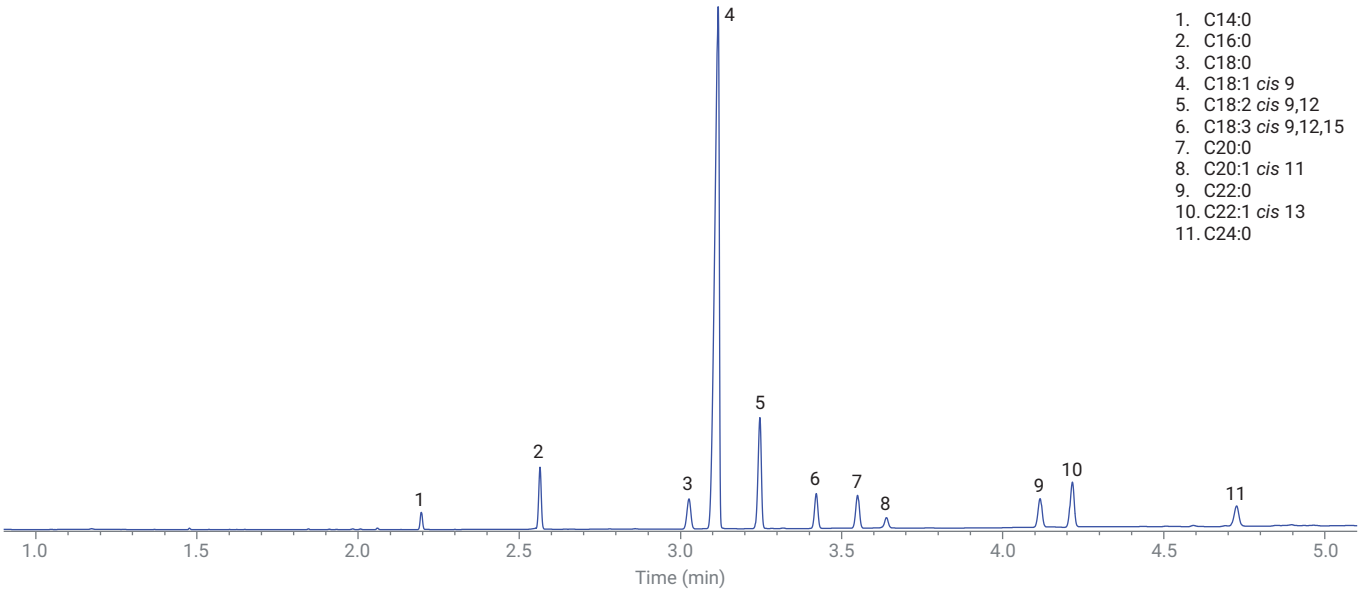

Figure 2. GC/FID chromatogram of rapeseed oil on a 20 m × 0.18 mm, 0.20 µm DB-FastFAME Intuvo column using Method 1 with helium as carrier gas (see Table 1).

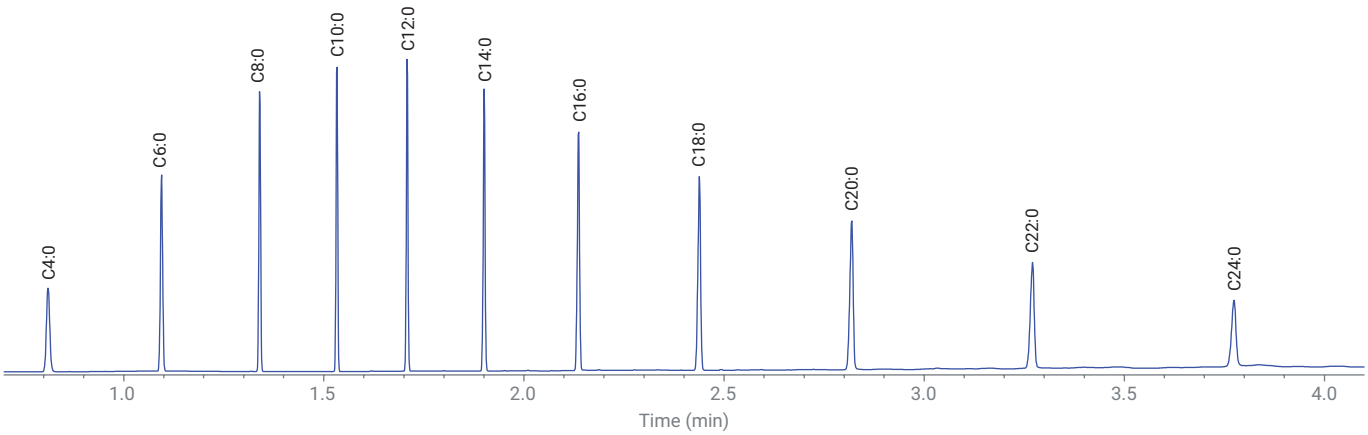

Figure 3. GC/FID chromatogram of the C4–C24 even carbon saturated FAME mixture on a 20 m × 0.18 mm, 0.20 µm DB-FastFAME Intuvo column using Method 2 with hydrogen as carrier gas (see Table 2).

For labs that validated their FAME methods with the traditional 37-component FAME mix, Figure 5 shows the chromatogram using the 20 m × 0.18 mm, 0.20 µm DB-FastFAME column on the Intuvo 9000 GC. The method with helium as carrier gas completely resolved all compounds within eight minutes.

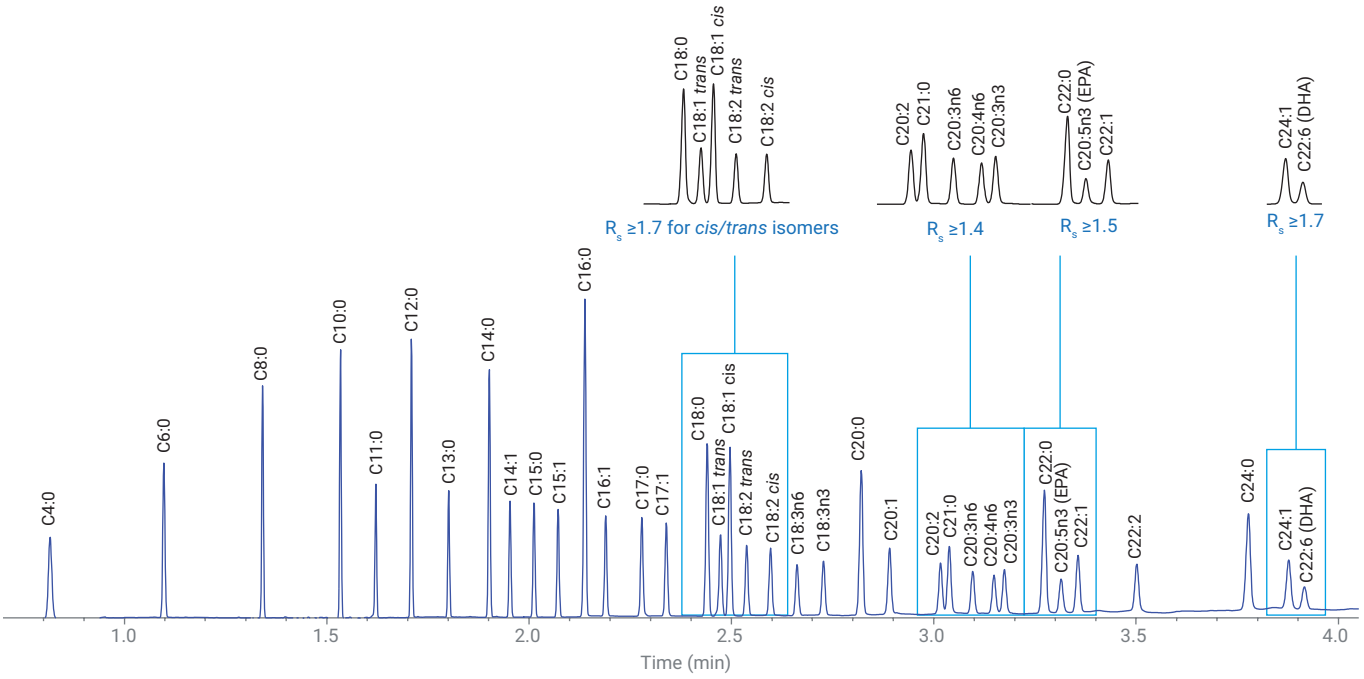

Figure 4. GC/FID chromatogram of the FAME 36 component mixture on a 20 m × 0.18 mm, 0.20 µm DB-FastFAME Intuvo column using Method 2 with hydrogen as carrier gas (see Table 2).

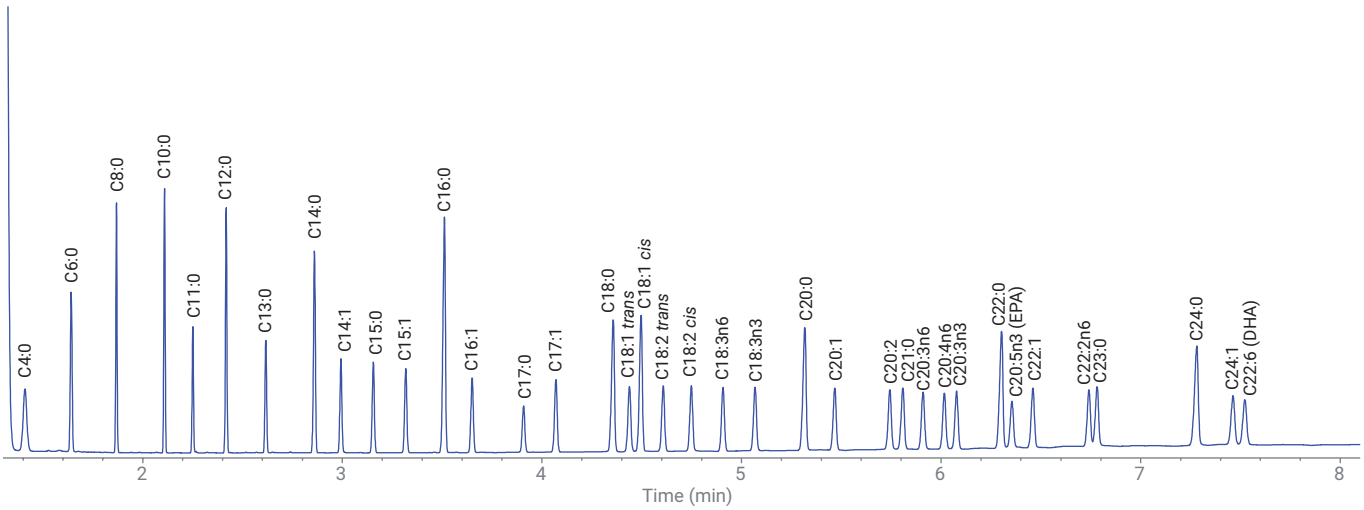

Figure 5. GC/FID chromatogram of 37-component FAMEs standard mixture on a 20 m × 0.18 mm, 0.20 µm DB-FastFAME Intuvo GC column using Method 3 with helium as carrier gas (see Table 3).

As expected, the use of hydrogen as a carrier gas provides a faster analysis with almost equivalent resolution. Figure 6 demonstrates that all compounds in the 37-component FAME mixture were well resolved, with an analysis time of less than 6.5 minutes with hydrogen as the carrier gas.

Conclusions

DB-FastFAME Intuvo GC columns can provide rapid and excellent separation for FAME mixtures. This Application Note demonstrates that all components in the FAME 36 component mixture, including AOAC critical pairs and key cis/*trans* fatty acid isomers, are well resolved on DB-FastFAME Intuvo GC columns within five minutes when using helium as the carrier gas. This work also demonstrates fast analysis of rapeseed oil. When using hydrogen as the carrier gas, the high-efficiency DB-FastFAME Intuvo GC column can reduce run times to under four minutes with all compounds baseline separated. An Intuvo 9000 GC paired with a DB-FastFAME GC column shows the possibility of low cost FAME analysis with high productivity and efficiency.

References

1. AOAC Official Methods of Analysis (**2000**), method Ce 2–66.
2. IUPAC, Standard Methods for the Analysis of Oils, Fats and Derivatives, Blackwell Scientific Publications, IUPAC Method 2.301.
3. National Food Safety Standard, Determination of fatty acids in food (食品中脂肪酸的测定), GB 5009.168-2016.
4. 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**.

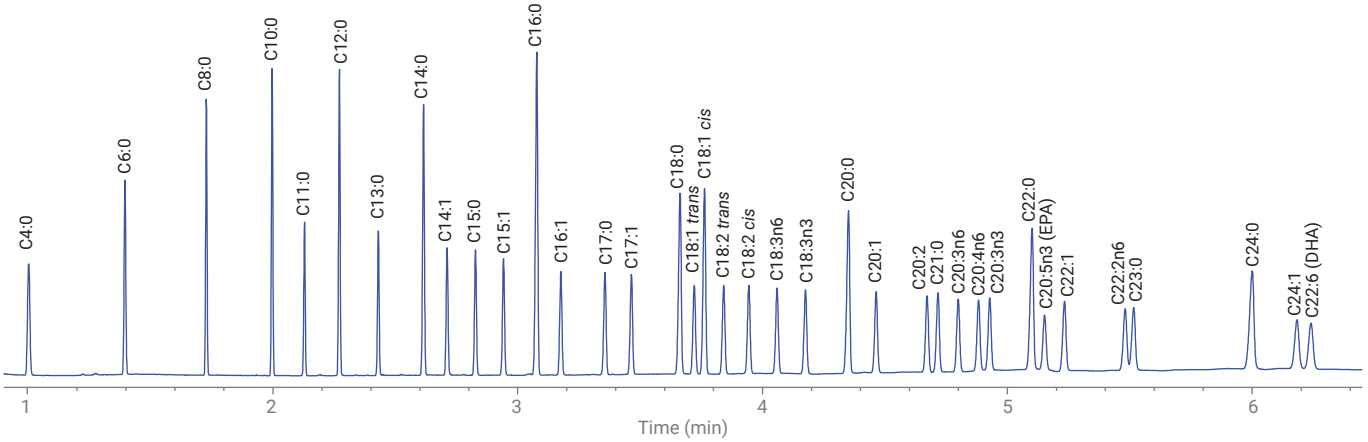

Figure 6. GC/FID chromatogram of 37-component FAME standard mixture on a 20 m × 0.18 mm, 0.20 µm DB-FastFAME Intuvo GC column using Method 4 with hydrogen as carrier gas (see Table 4).

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