Rapid Separation of trans/cis Fatty Acid Methyl Esters with Agilent DB-FastFAME GC Column

Julian de la Mata¹, Gustavo Serrano², Phil

Madrid, Madrid, Spain, ²2850 Centerville Rd, Wilmington, DE 19808, Agilent Technologies, ³412 Ying Lun Road, Shanghai 200131, China, Agilent

17th Euro Fed Lipid POSTER # ANAUL-019



Introduction

- The analysis of oils, fat, and fat-containing food is a common task in governmental, quality control (QC), contract research organizations 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 food.
- · 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.
- Traditionally, long GC columns (100 m) and long analysis times (more than 75 min), are required to achieve good FAME separations
- However, this leads to high analysis costs and low productivity.
- · The new Agilent J&W DB-FastFAME GC columns with a specially engineered high-content cyanopropyl phase was designed for the fast separation of FAME mixtures, including cis/trans isomer separations, to meet the requirements of regulatory methods.
- This research work demonstrates rapid analysis of FAME mixture using several DB-FastFAME column configurations.

Experimental

Chemicals and Standards

- · The 37-FAME mix was purchased from a local supplier.
- · Additional individual FAME standards were purchased individually and added to the 37-FAME mix to obtain the 57 FAME mixture

Instrumentation

The analyses were performed using an Agilent 7890 GC equipped with a flame ionization detector (FID).

DB-FastFAMF, 30 m x 0.25 mm x 0.25 um.

Sample introduction was done using an Agilent 7693A automatic liquid sampler with 5 µL syringe.

GC Conditions

Method	1
Column	

Column	(p/n G3903-63011)
Carrier	Helium, 13.8 psi, constant pressure mode
Oven	50 °C (1 min), 25 °C/min to 175 °C, 4 °C/min to 230 °C (5 min)
Inlet	Split/Splitless, 250 °C, split ratio 50:1
FID	260 °C, H ₂ : 40 mL/min, Air: 400 mL/min
Injection	1 μL
Method 2	
Column	DB-FastFAME, 60 m x 0.25 mm x 0.25 μm (p/n G3903-63012)
Carrier	Helium, constant pressure, 35 psi
Oven	80 °C (1.5 min), 45 °C/min to 205 °C (11 min); 12 °C/min to 235 °C (10 min)
Inlet	260 °C, split/splitless mode split ratio: 15:1
FID	260 °C, H ₂ : 40 mL/min, Air: 400 mL/min
Injection	1 μL
Method 3	
Column	DB-FastFAME, 90 m x 0.25 mm x 0.25 μm

(p/n G3903-63013)

Carrier Helium, constant pressure, 40 psi 75 °C (1.5 min), 30 °C/min to 200 °C (5 min); Oven 2.5 °C/min to 206 °C (1.5 min), 12 °C /min to 230 °C (30 min) Inlet

260 °C, split/splitless mode split ratio: 15:1 260 °C, H₂: 40 mL/min, Air: 400 mL/min

Injection $1 \mu L$

FID

Results

- Figure 1 shows the separation of a traditional 37-FAME mix using a 30-m DB-FastFAME in under 22 min. This column is useful for most nutritional FAME analysis, including cis/trans isomers.
- Figure 2 shows the separation of the same mix, plus additional conjugated Linoleic Acid (CLA, C18:2 c9, t11, and C18:2 t10,c12) with a 60m DB-FastFAME under 25 minutes. This column is ideal for separation of cis/trans isomers in the C18:2 and C18:3 region, including CLA isomers.
- Finally, figure 3 shows the separation of a 57-FAME mix, including positional cis/trans isomers in the C18:1, C18:2 and C18:3 region with a 90-m DB-FastFAME GC column.

This high-resolution column provides an Rs value of 1.4 for the challenging C18:1 trans 11 and C18:1 cis 6 pair, make it ideal for the proper quantification of trans fat in food samples.

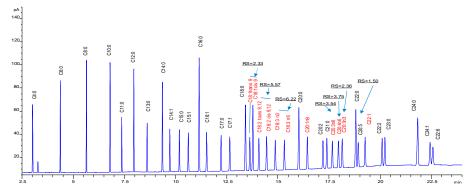


Figure 1. GC/FID chromatogram of 37-component FAME standard mixture on a 30m x 0.25mm i.d. x 25 m Agilent J&W DB-FastFAME using Method 1

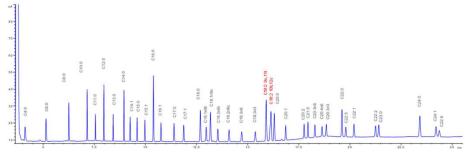


Figure 2. GC/FID chromatogram of 39-component FAME standard mixture on a 60m x 0.25mm i.d. x 25 m Agilent J&W DB-FastFAME using Method 2

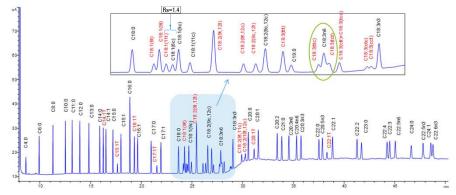


Figure 3. GC/FID chromatogram of 57-component FAME standard mixture on a 90m x 0.25mm i.d. x 25 m Agilent J&W DB-FastFAME using Method 3

Conclusions

- DB-FastFAME GC columns can provide rapid and excellent separation of FAME mixtures, with several dimensions available for specific applications
- The 30m DB-FastFAME is ideal for most nutritional FAME analysis, including cis/trans separations in 22
- The 60-m DB-FastFAME is ideal for complex FAME analysis, including CLA FAME isomers in under 25 min,
- and the 90-m DB-FastFAME offers the highest resolution for challenging positional cis/trans isomers in under 50 min.

References

- 1.AOAC Official Methods for 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