Application Note

Food and Beverage Testing

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Fatty Acid Methyl Ester Analysis Using the Agilent 8850 GC System

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Abstract

This application note presents the analysis of fatty acid methyl esters (FAMEs) using an Agilent 8850 gas chromatograph (GC) system. Leveraging the 8850's rapid temperature programming, 20-meter and 30-meter Agilent J&W DB-FastFAME GC columns enabled the fast separation of 37 common FAMEs within 10 and 15 minutes, respectively. To analyze more complex FAMEs samples, including cis-*trans* and positional isomers of linoleic and linolenic acid methyl esters, a 90-meter Agilent DB-FastFAME GC column, and a 100-meter Agilent J&W HP-88 column were employed, using a conventional temperature ramp on the 8850 GC platform. Additionally, helium and nitrogen carrier gases were evaluated across the three DB-FastFAME analytical columns to assess their impact on the target analysis. Method performance, including key compound pairs resolution, precision, and analysis speed, was evaluated.

Introduction

Fatty acids are components of fat and are an essential component of a healthy diet. They are found in a variety of foods, including oily fish, nuts, seeds, and vegetable oils. Fatty acids can be categorized as saturated fat, monounsaturated fat, polyunsaturated fat (including omega-3 and omega-6 fatty acids), and trans fat. Unsaturated fatty acids play a beneficial role in maintaining heart and vascular performance. However, artificial trans fatty acids, found in processed foods, should be strictly limited. The measurement of fatty acids in food plays an important role in:

- **Nutritional assessment:** Analyzing the omega-3/omega-6 ratio for dietary nutrient balance
- **Safety regulation:** Identifying health risks such as trans fatty acids
- **Quality control:** Detecting oil adulteration and process defects
- **R&D support:** Providing data support for the development of functional foods

Techniques available for fatty acid determination in food include gas chromatography, liquid chromatography, and spectroscopic techniques. Each technique has its own advantages, disadvantages, and applicable scenarios. For example, liquid chromatography is suitable for analyzing heat-labile fatty acids, but it suffers from low resolution. Fluorescence spectroscopy is simple to operate but susceptible to matrix effects and poor specificity. Gas chromatography is the most used technique for fatty acid measurement. Fatty acids in food primarily exist in the form of triglycerides. Before analysis, triglycerides must be extracted, saponified, and methylated to the corresponding FAMEs. FAMEs are less polar and more volatile than fatty acids, making them suitable for analysis on gas chromatography platforms. Polar columns, such as those with polyethylene glycol and cyanopropyl siloxane stationary phases, are primarily used for the FAMEs analysis. Polyethylene glycol stationary phases are good at analyzing simple fatty acid mixtures and are not suitable for the analysis of *cis-trans* isomers. A column coated with high-content cyanopropyl phase is recommended in different analytical methods for FAMEs analysis. However, an analysis based on this phase type usually takes more than 70 minutes to finish, and the retention time (RT) stability is not very good. The Agilent J&W DB-FastFAME column features a modified cyanopropyl phase to accelerate the analysis and improve repeatability.

Agilent offers DB-FastFAME columns in different dimensions; the 20-meter and 30-meter sizes can help improve the analysis speed for 37 representative FAMEs in food. Previous work demonstrated the analysis speed improvement using the conventional oven ramp rate on the two columns.[1] With the launch of the Agilent 8850 GC, rapid temperature programming can be achieved in an air bath oven. This application note demonstrates the fast analysis of 37 FAMEs using nitrogen (N_2) and helium (He) carrier gases on the 8850 GC. Meanwhile, more complex FAME mixtures including 37 FAMEs and 15 *trans* FAMEs were analyzed on a 90-meter DB-FastFAME column and a 100-meter Agilent J&W HP-88 column to demonstrate the 8850 GC performance in *cis/trans* FAMEs and position isomers analysis.

Experimental

Chemicals

- **FAME mixture 1:** C4–C24 FAMEs with concentration varying from 200 to 600 µg/mL in iso-octane, 37-component (CDAA-M-252795-DZ-1.2ml)
- **FAME mixture 2:** trans FAME mixture, 100 µg/mL each in iso-octane, 8-component (CDAA-M-259004-DA-1ml)
- **FAME mixture 3:** 10 mg/mL linolenic acid methyl ester mixture in CH_2CL_2, 8-component (CRM47792)
- **FAME mixture 4:** 10 mg/mL linoleic acid methyl ester mixture in CH_2CL_2, 4-component (CRM47791)

All chemicals were purchased from ANPEL Laboratory Technologies (Shanghai) Inc.

Mixture 1 was diluted fivefold with iso-octane for fast separation on 20 m and 30 m Agilent J&W DB-FastFAME columns.

Mixtures 1 to 4 were blended for *trans/cis* FAME analysis on 90-meter DB-FastFAME and 100-meter HP-88 columns.

Instrumentation

An 8850 GC system equipped with a split/splitless inlet and a flame ionization detector (FID) was used for the analysis. An Agilent 7650A automatic liquid sampler (ALS) (part number G4567A) was applied for sample injection.

The methods performed on the four analytical columns using He and N_2 carrier gases are listed in Table 1. The settings on inlet and detector temperatures are the same. The column head pressure and the oven temperature program were optimized based on the column type and carrier gas. Data were acquired and processed using Agilent OpenLab CDS 2.8. The list of consumables used is shown in Table 2.

Table 1. 8850 GC instrument parameters.

Parameter	Value			
Split/Splitless Inlet	220 °C, split ratio: 20:1~100:1			
FID	240 °C H_2: 30 mL/min Air: 400 mL/min Makeup (N_2): 20 mL/min			
Column Type	**20 m Agilent J&W DB-FastFAME**	**30 m Agilent J&W DB-FastFAME**	**90 m Agilent J&W DB-FastFAME**	**100 m Agilent J&W HP-88**
Oven Program and Column Head Pressure Settings (He-Based Methods)				
Carrier Gas	Helium			
Column Head Pressure	28 psi, CP*	27 psi, CP	34 psi (1.5 min), 6 psi/min to 40 psi, RP*	40 psi, CP
Oven Program	60 °C (0.5 min), 300 °C/min to 175 °C (0.32 min), 23 °C/min to 210 °C (1 min), 23.5 °C/min to 220 °C (1 min), 60 °C/min 250 °C (3 min)	60 °C (0.49 min), 300 °C/min to 175 °C (0.49 min), 15 °C/min to 210 °C (2.5 min), 14.5 °C/min to 240 °C (3 min)	75 °C (1.5 min), 150 °C/min to 200 °C (20 min), 2 °C/min to 208 °C (0.8 min), 9 °C/min to 235 °C (21 min)	100 °C (13 min), 10 °C/min to 180 °C (6 min), 1 °C/min to 200 °C (20 min), 4 °C/min to 250 °C (2 min)
Oven Program and Column Head Pressure Settings (N_2-Based Methods)				
Carrier Gas	Nitrogen			
Column Head Pressure	20 psi	14 psi	30 psi (1.5 min), 6 psi/min to 36 psi, RP	
Oven Program	60 °C (0.58 min), 250 °C/min to 175 °C (0.37 min), 19.6 °C/min to 210 °C (1.6 min), 20.1 °C/min to 225 °C (2 min), 180 °C/min 250 °C (2 min)	60 °C (0.64 min), 130 °C/min to 175 °C (1.3 min), 8 °C/min to 210 °C (5.4 min), 50 °C/min to 250 °C (4 min)	75 °C (1.5 min), 150 °C/min to 200 °C (20 min), 3 °C/min to 208 °C (8 min), 9 °C/min to 235 °C (18 min)	

* CP stands for constant pressure; RP is for ramped pressure. The column head pressure of the 20-meter and 30-meter columns using nitrogen carrier gas may need adjustment to get similar resolution considering column batch difference.

Table 2. Consumables used in FAMEs analysis.

Category	Agilent Part	Part Number
Inlet Septa	Hi-temp/low bleed/nonstick septa	5183-4757
Inlet Liner	Ultra-inert, low pressure drop split liner with glass wool	5190-2295
ALS Syringe	Agilent Gold Standard, 23–26 s tapered needle	5181-1273
Column 1	Agilent J&W DB-FastFAME, 20 m × 0.18 mm, 0.20 µm, custom 5 inch format	100-2000
Column 2	Agilent J&W DB-FastFAME, 30 m × 0.25 mm, 0.25 µm	G3903-63011
Column 3	Agilent J&W DB-FastFAME, 90 m × 0.25 mm, 0.25 µm, custom 5 inch format	100-2000
Column 4	Agilent J&W HP-88 GC column, 100 m, 0.25 mm, 0.2 µm	112-88A7E

Results and discussion

Fast analysis of 37 fatty acid methyl esters on short columns

The 37 FAMEs mimic the fatty acid composition of many food samples, including most of the important saturated, monounsaturated, and polyunsaturated FAMEs. They are well-established and widely accepted reference materials.

A 20 m × 0.18 mm id, 0.2 µm DB-FastFAME column and a 30 m × 0.25 mm id, 0.25 µm DB-FastFAME column were used to demonstrate the high-speed analysis of 37 FAMEs on the 8850 GC. The oven ramp program and column flow rate were based on previous work and further optimized for the 8850 GC. Both He and N_2 carrier gas-based methods were developed considering the increasing alternative carrier gas need and lab operation habits in different regions.

The GC-FID chromatograms obtained on the two columns using different carrier gases are shown in Figures 1A, 1B, 2A, and 2B. All compounds were well resolved in the He methods. Three compound pairs that cannot be baseline-separated are shown in black squares. Among them, the peaks of c22:2n6 and c23:0 had the lowest resolution of 1.27 and 1.31 on the 20-meter and 30-meter columns, respectively, using the He method. The AOAC International Method 996.06[2] requires separating the FAME pair of adjacent peaks of C18:3 and C20:1 and the FAME trio of adjacent peaks of C22:1, C20:3, and C20:4 with a resolution of 1.0 or greater. This requirement is mainly recommended for high-content cyanopropyl

stationary phases. The six probe FAMEs eluted in different orders on the DB-FastFAME column, as marked by the yellow squares in the chromatogram. Their separation exceeded the resolution requirement easily due to the DB-FastFAME column's unique selectivity. The analysis times of the He methods were less than 7 minutes and 10 minutes on the two columns. The analysis speed was five to eight times faster than that of conventional methods.

The late-eluted compounds in the N_2 method have wider peak shapes, which impacted their resolution with adjacent peaks. Switching to N_2 carrier gas on the 20-meter column, the resolutions of C22:2n6/C23:0 and C24:1/C22:3n6 decreased from 1.27 and 1.36 to 1.1 and 1.01, respectively. The analysis time increased about 30%. To improve the N_2 method resolution, the analysis speed should be lowered. Figure 2B shows the chromatogram generated on the 30-meter column using N_2 carrier gas under a slower column flow rate and

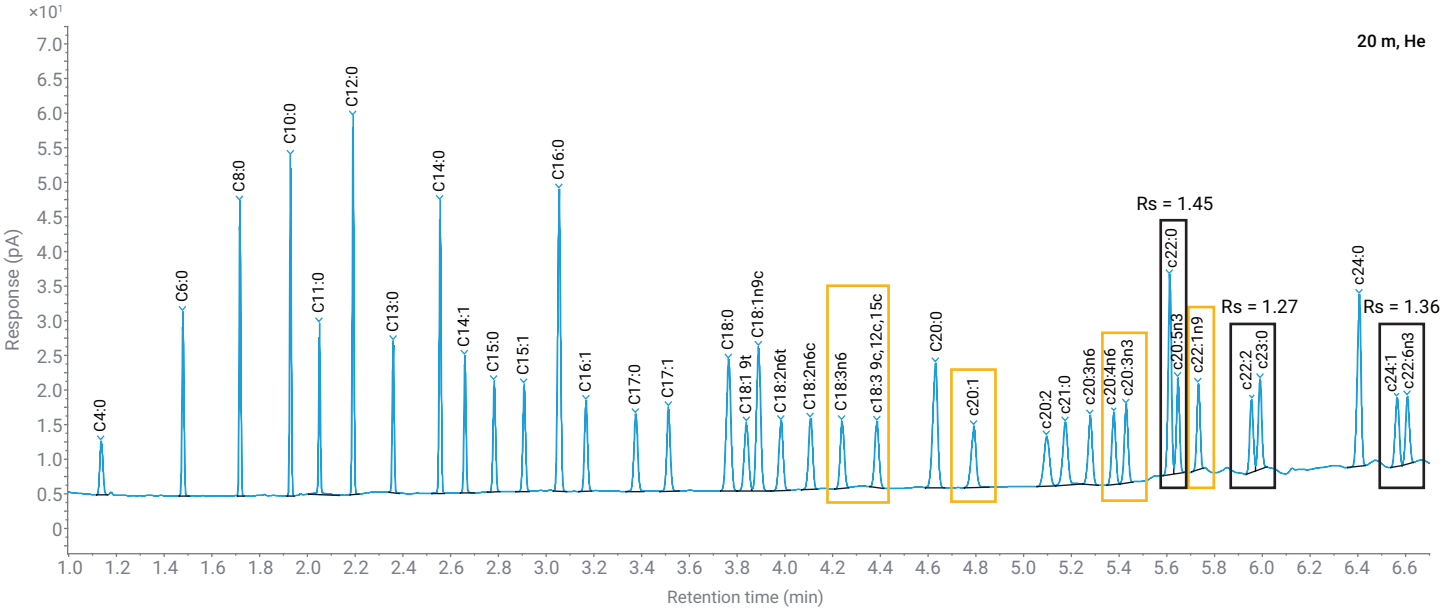

Figure 1A. The chromatogram of 37 FAMEs on a 20-meter Agilent J&W DB-FastFAME column using He carrier gas.

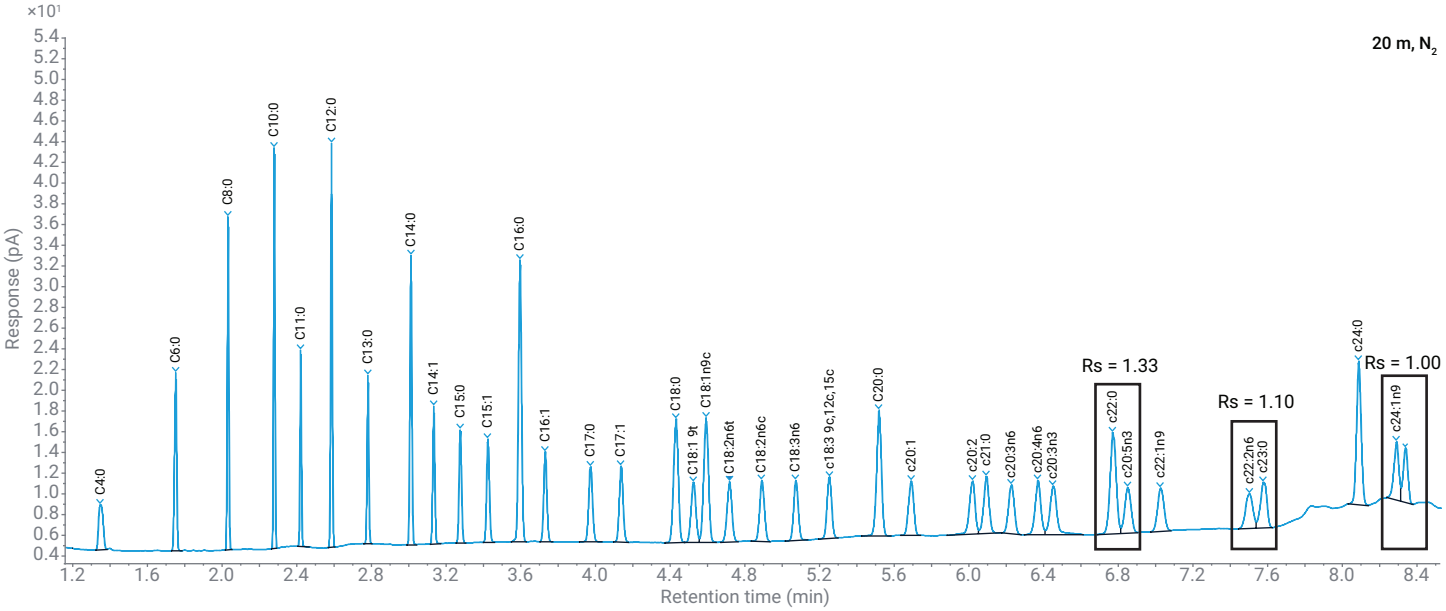

Figure 1B. The chromatogram of 37 FAMEs on a 20-meter Agilent J&W DB-FastFAME column using N_2 carrier gas.

oven program. The resolution of c22:2n6 and c23:0 in the N_2 method for the 30-meter column was 1.30, equivalent to 1.31 in the He method. The analysis speed delivered by the N_2 method on the 30-meter column was 50% slower than the He method (14.5 minutes versus 9.5 minutes).

Although high-speed FAME analysis can be achieved on short DB-FastFAME columns using both He and N_2 carrier gases, to obtain better resolution and higher productivity, the He-based method is the better choice.

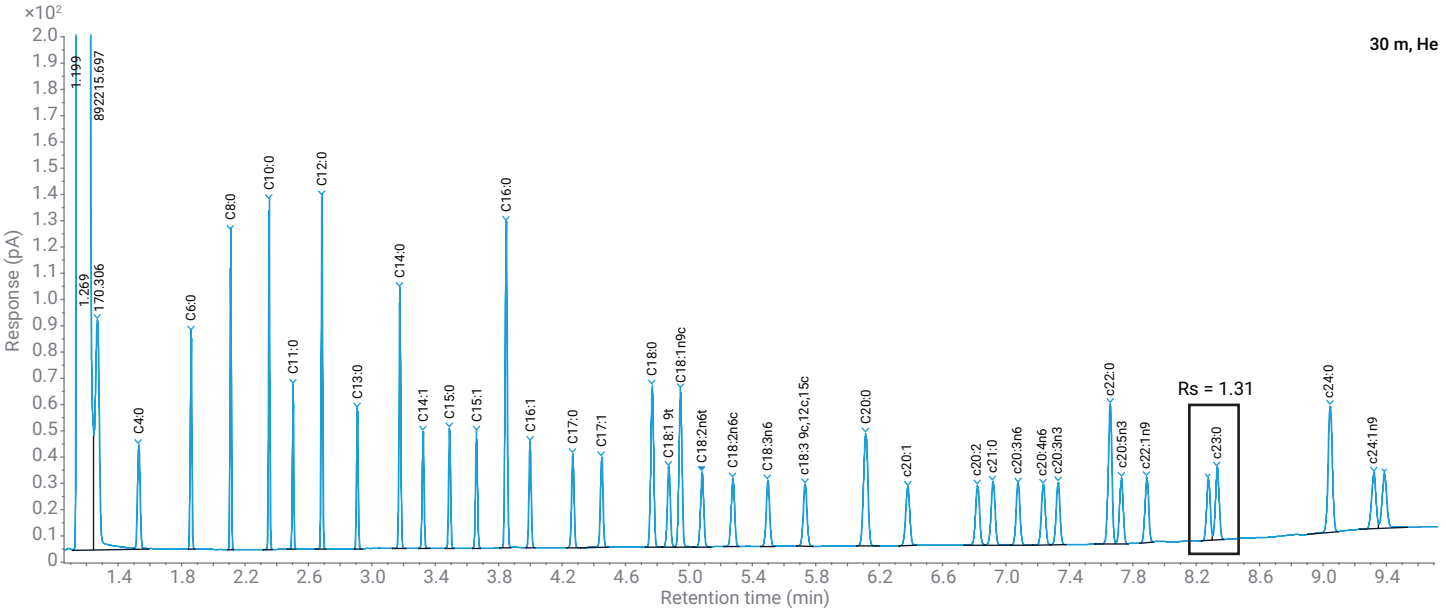

Figure 2A. The chromatogram of 37 FAMEs on a 30-meter Agilent J&W DB-FastFAME column using He carrier gas.

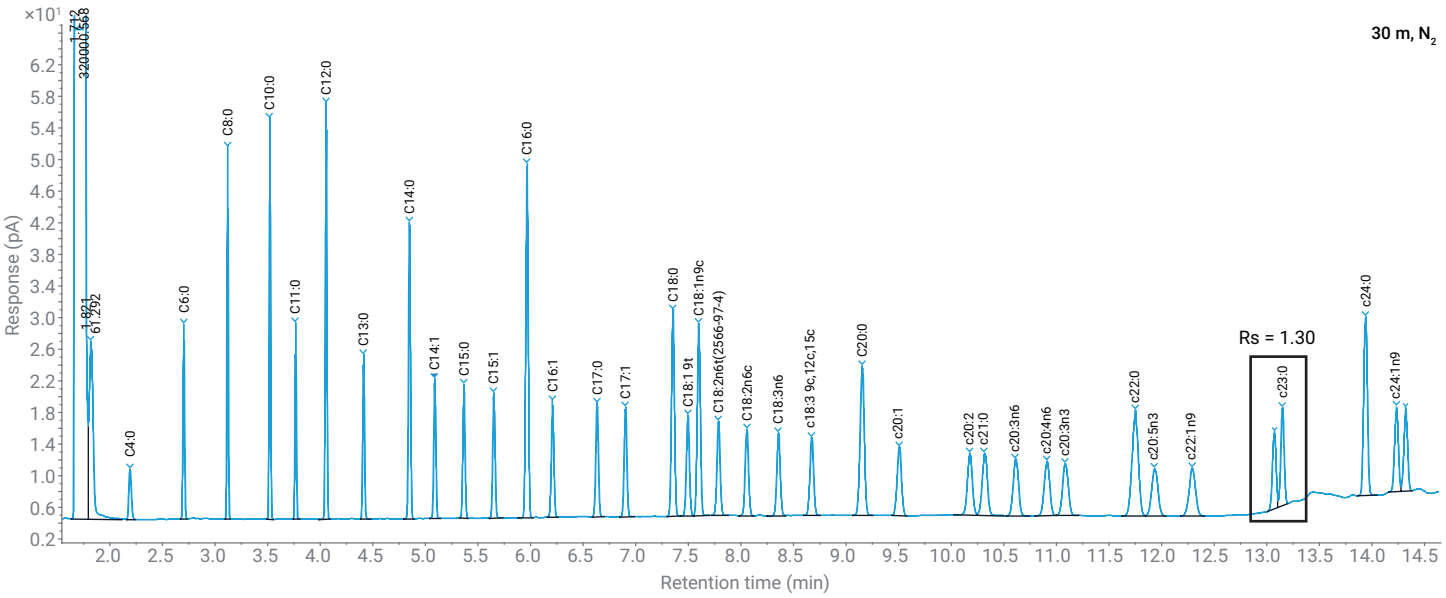

Figure 2B. The chromatogram of 37 FAMEs on a 30-meter Agilent J&W DB-FastFAME column using N_2 carrier gas.

The RT and response precision of the four fast methods were evaluated based on seven consecutive injections of 37 FAMEs. The analyte amount loaded on the column ranged from 0.4 to 1.2 ng. As shown in Figure 3, the area repeatability is from 0.2 to 3.8%. The late-eluted compounds showed a higher area %RSD (2 to 3.8%) because of the baseline impact on their peak integration. Under the high oven temperature, the baseline of the DB-FastFAME column is not as flat as that under low temperature. The fluctuating baseline influenced the start or end point of integration on those late eluents and made the integration unrepeatable. The variability in peak areas was reflected in the slightly higher response %RSD. For real sample analysis, the fatty acid amount is generally much higher than the test standard. Their response is much bigger and less likely to be impacted by the baseline fluctuation. Accordingly, the quantitation precision will be better.

The RT precision of the four methods was in the range of 0.01 to 0.07% (Figure 4)—good precision considering the analysis speed, which is the guarantee to accurate qualification.

It should be emphasized the fast analysis is suitable for the characterization of classical samples such as vegetable oils from corn, soybean, maize, etc. For samples of animal origin, such as dairy products and omega-3 acids in fish oil, the fast analysis method can be applied to the quality control process. For the more complex samples that require *cis/trans* fatty acid and positional isomer (i.e., double-bond position isomer) analysis, it is better to seek to the assistance of a long polar column and a slower temperature program for satisfactory resolution.

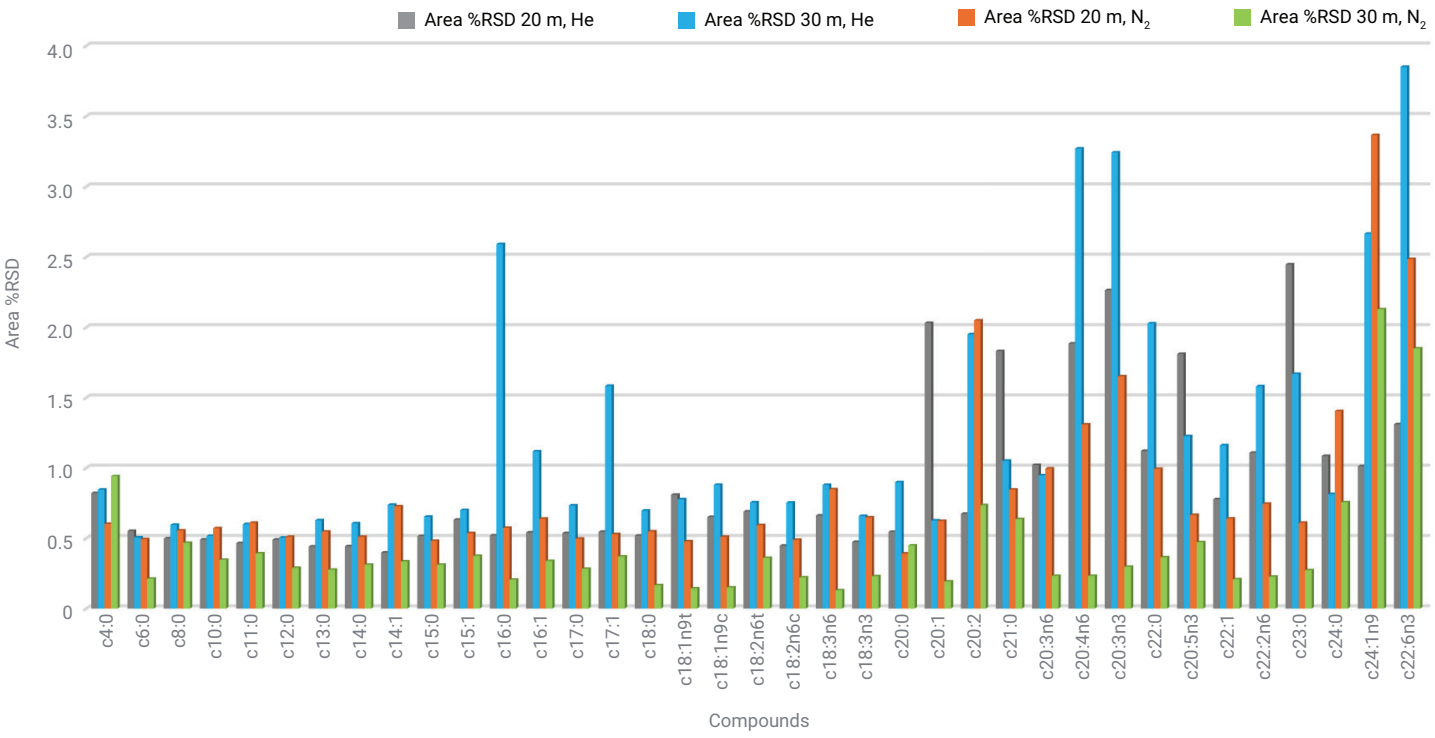

Figure 3. Area precision on 20-meter and 30-meter Agilent J&W DB-FastFAME columns using helium and nitrogen carrier gases.

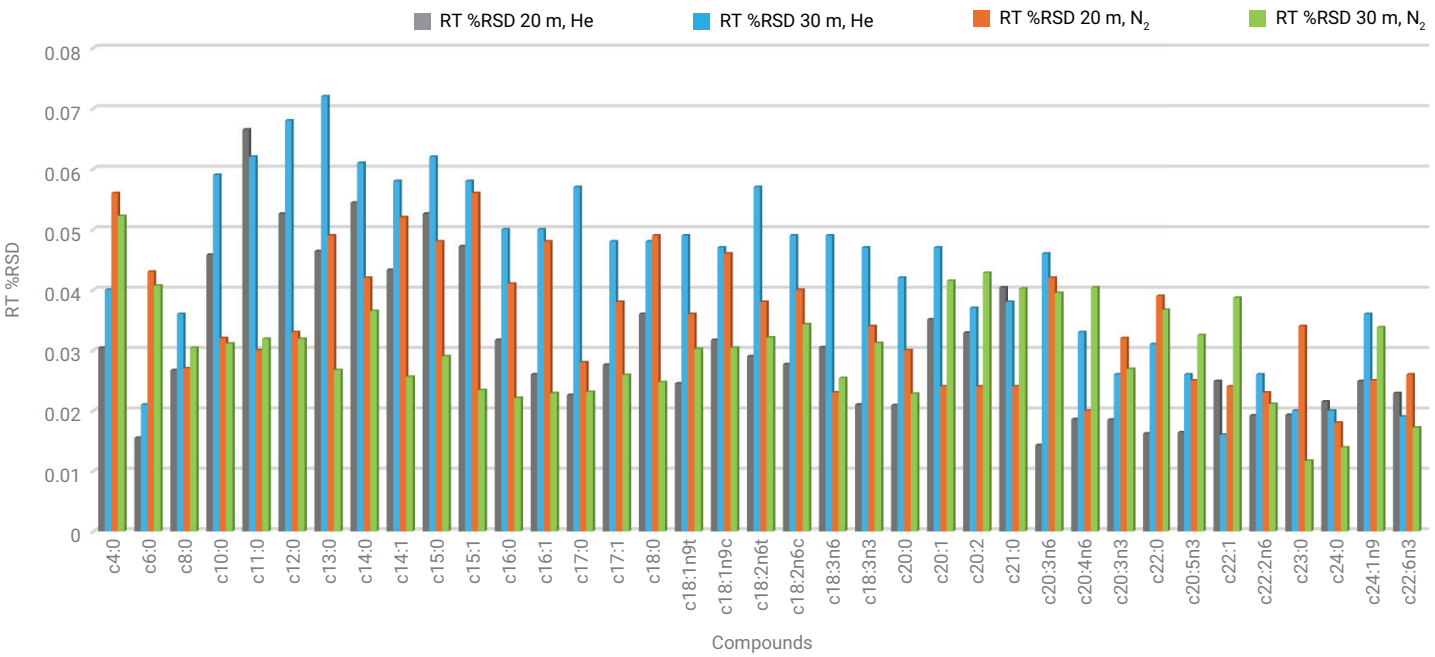

Figure 4. Retention time precision on 20-meter and 30-meter Agilent J&W DB-FastFAME columns using helium and nitrogen carrier gases.

Analysis of complex *trans*/*cis* fatty acids on 90-meter DB-FastFAME and 100-meter HP-88 columns

A comprehensive fatty acid analysis requires resolving not only saturated and unsaturated fatty acids but also the multiple *trans/cis* fatty acid isomers, such as linolenic acid (C18:3) and linoleic acid (C18:2) isomers. The GB 5009.168-2016[3] and GB 5009.257-2016[4] methods in China's Catalogue of National Food Safety Standards require analyzing 52 fatty acids in a food matrix. C18:2 and C18:3 isomers and 37 FAMEs are in the target list. Short DB-FastFAME columns are not designed for this application. Traditionally, such separation is run on long high-content cyanopropyl phase columns such as the HP-88 and Agilent J&W CP-Sil 88 columns. Here, a 90-meter DB-FastFAME column was applied for the target separation on the Agilent 8850 GC. The resolution, analysis speed, and method precision were evaluated.

The mixtures of 37 FAMEs and 15 *trans*-FAMEs were separated using an optimized oven temperature program under He and N_2 carrier gases. A ramp in column head pressure was developed to match the fast ramp segment in the oven program. The analysis time is under 50 minutes using the He method and slightly longer (< 55 minutes) using the N_2 method. Figure 5A and Figure 5B show the chromatograms and detailed resolution of C18:2 and C18:3 isomers on the 90-meter DB-FastFAME column. It is difficult to separate all adjacent peaks with baseline-level resolution. The target is to have most of *cis* and *trans* FAMEs separated with a resolution >1.0. Only one compound pair of C18:3 (6c,9c,12c) (*cis*-) and C18:3 (9t,12t,15c) (*trans*-) could not meet this resolution in the He method. The FAME trio of C18:3 (9t,12t,15c), C18:3 (6c,9c,12c) and C18:3 (9t,12c,15c) could not meet this goal in the N_2 method.

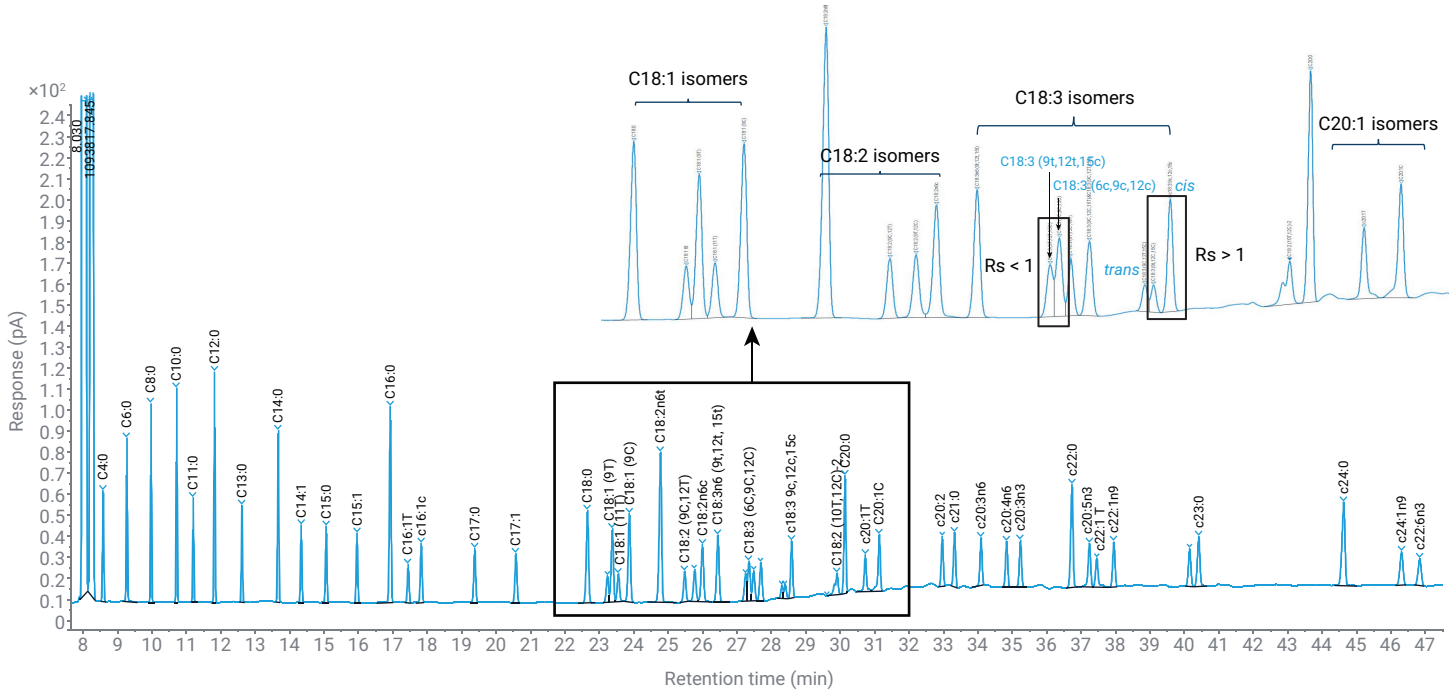

Figure 5A. Analysis of 52 FAMEs on the 90-meter Agilent J&W DB-FastFAME column (He).

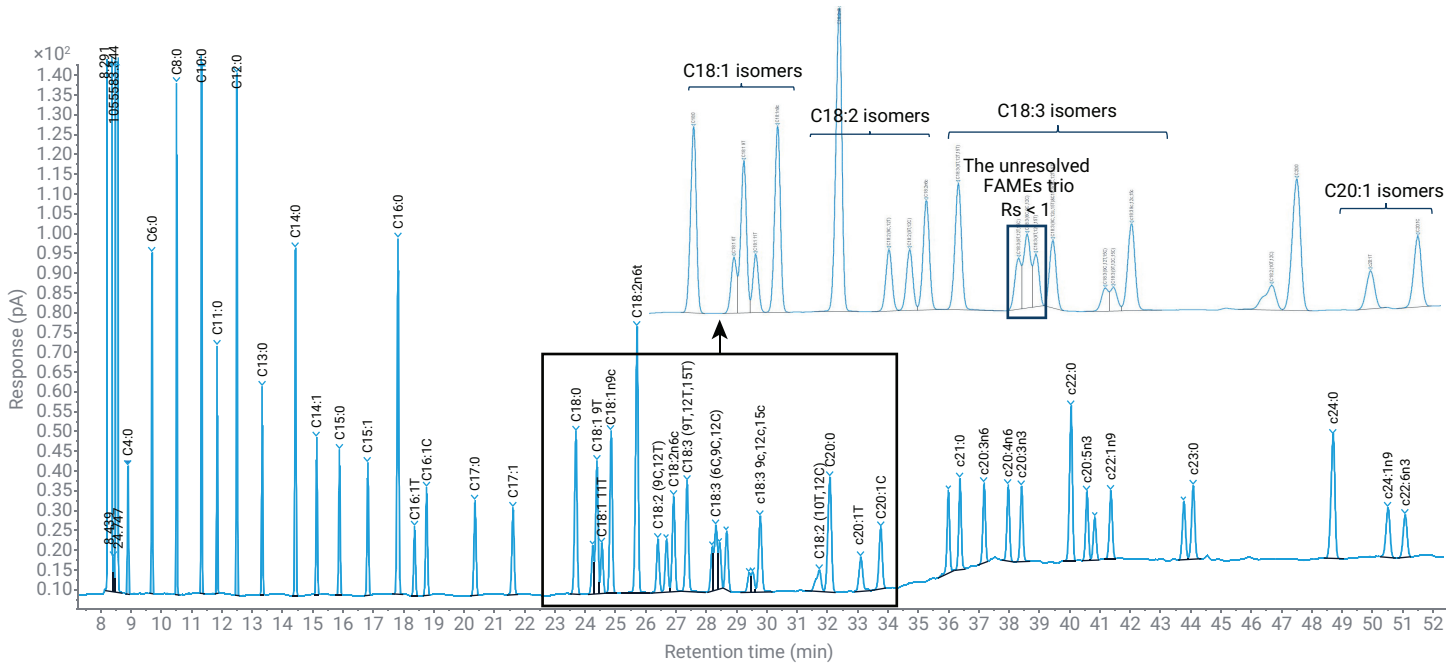

Figure 5B. Analysis of 52 FAMEs on the 90-meter Agilent J&W DB-FastFAME column (N_2).

For comparison, the same standard was analyzed on a 100-meter HP-88 column in the same 8850 GC system. Figure 6 shows the chromatogram on 100-meter HP-88 columns using He carrier gas. The analysis took around 75 minutes. Similar to DB-FastFAME column, the HP-88 type column faced a challenge with the C18:3 isomer resolution. There is one pair of *cis/trans* FAME coelution and insufficient resolution for the FAME trio of C18:3 (9c,12c,15t), C18:3 (6c,9c,12c) and C18:3 (9c,12t,15t) on the HP-88 column. The selectivity difference of the two test columns was exemplified in compound elution order and resolution difference. As shown in Figure 7, the HP-88 column could not resolve C20:0 with one of C18:3 isomers, C18:3 (9t,12c,15t). The coeluted peak was marked by the red box in the top chromatogram. In comparison, these two compounds could be well separated on a DB-FastFAME column, with C20:0 eluting far behind C18:3 (9t,12c,15t). All other compounds could be well separated on both columns. The chromatogram comparison demonstrated that the 90-meter DB-FastFAME column could achieve equivalent separation of the 52 FAMEs targeted by the GB 5009.168-2016 and GB 5009.257-2016 methods in a shorter time compared to the conventional high-content cyanopropyl phase column.

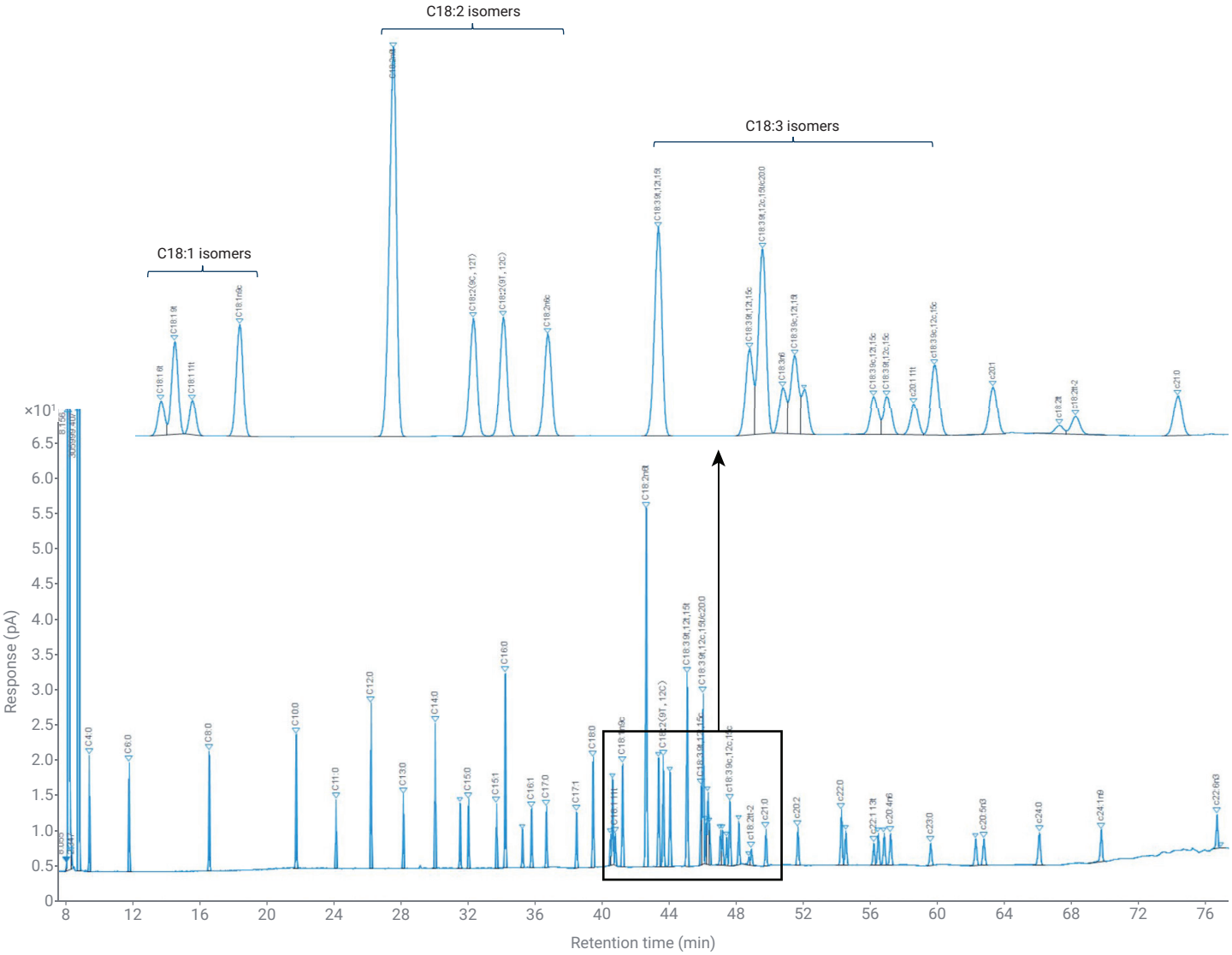

Figure 6. 52 FAMEs analysis on 100m HP-88 column (He).

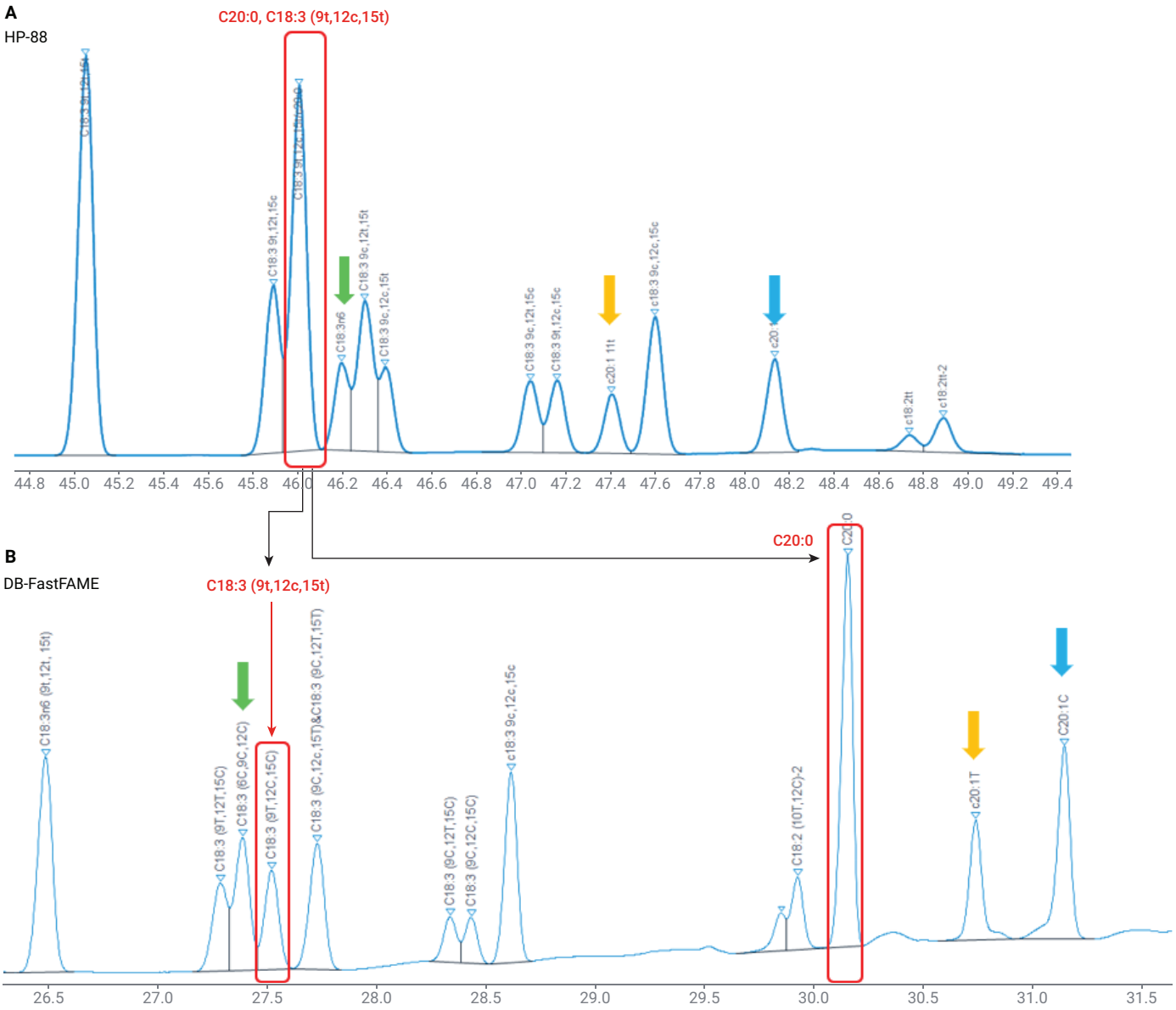

Figure 7. C18:3 isomer separation on the 100-meter Agilent J&W HP-88 (A) and 90-meter Agilent J&W DB-FastFAME (B) columns. The peaks labeled by the arrows of the same color belonged to the same compounds that eluted in different orders on the DB-FastFAME and HP-88 columns.

The analysis precision on the 90-meter DB-FastFAME column was evaluated by running six replicates of 52 FAME mixtures. The area repeatability was in the range of 0.22 to 2.7% (Figure 8.) RT repeatability was in the range of 0.005 to 0.041% (Figure 9). The response and RT precision were excellent and equivalent to the previous results on the 8890 GC, indicating that the 8850 GC can deliver accurate and stable control of oven temperature, inlet pressure, and detector flow rates across the long run time, which ensures reliable identification for complex FAME samples.

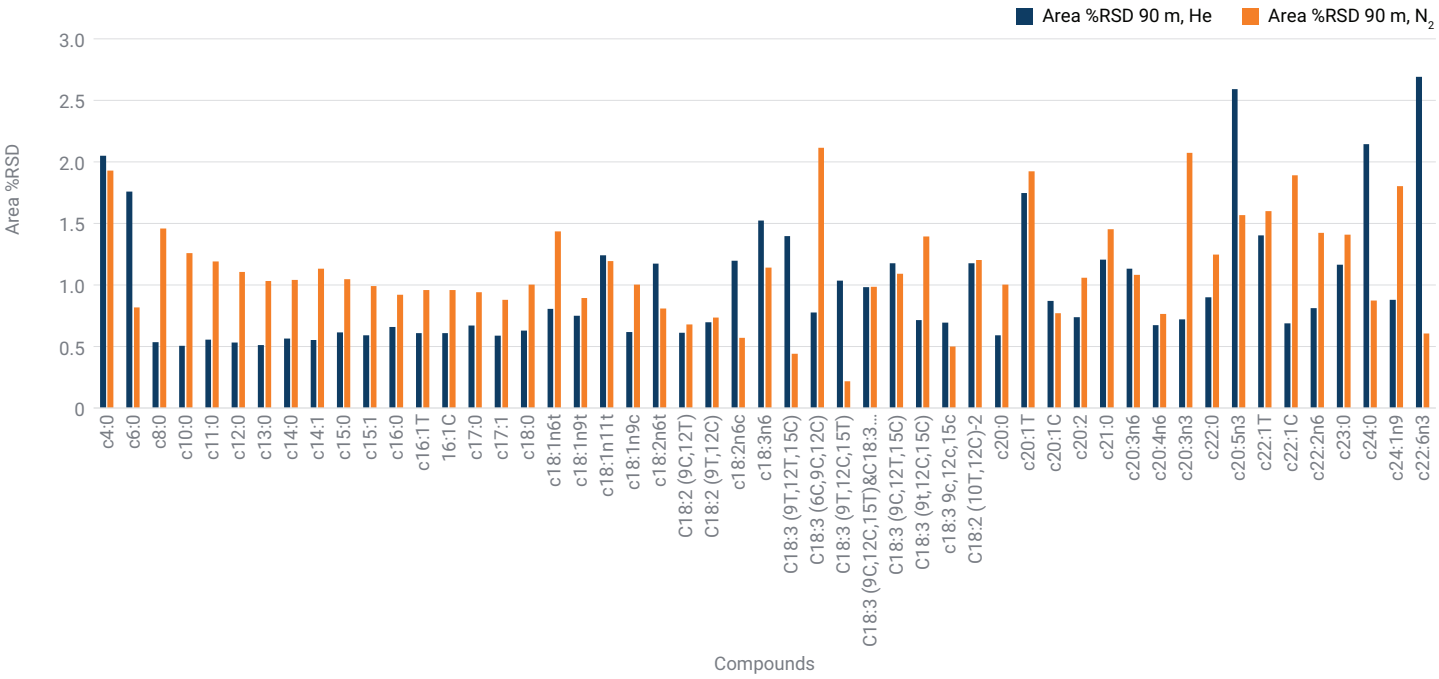

Figure 8. Response precision on the 90-meter Agilent J&W DB-FastFAME column.

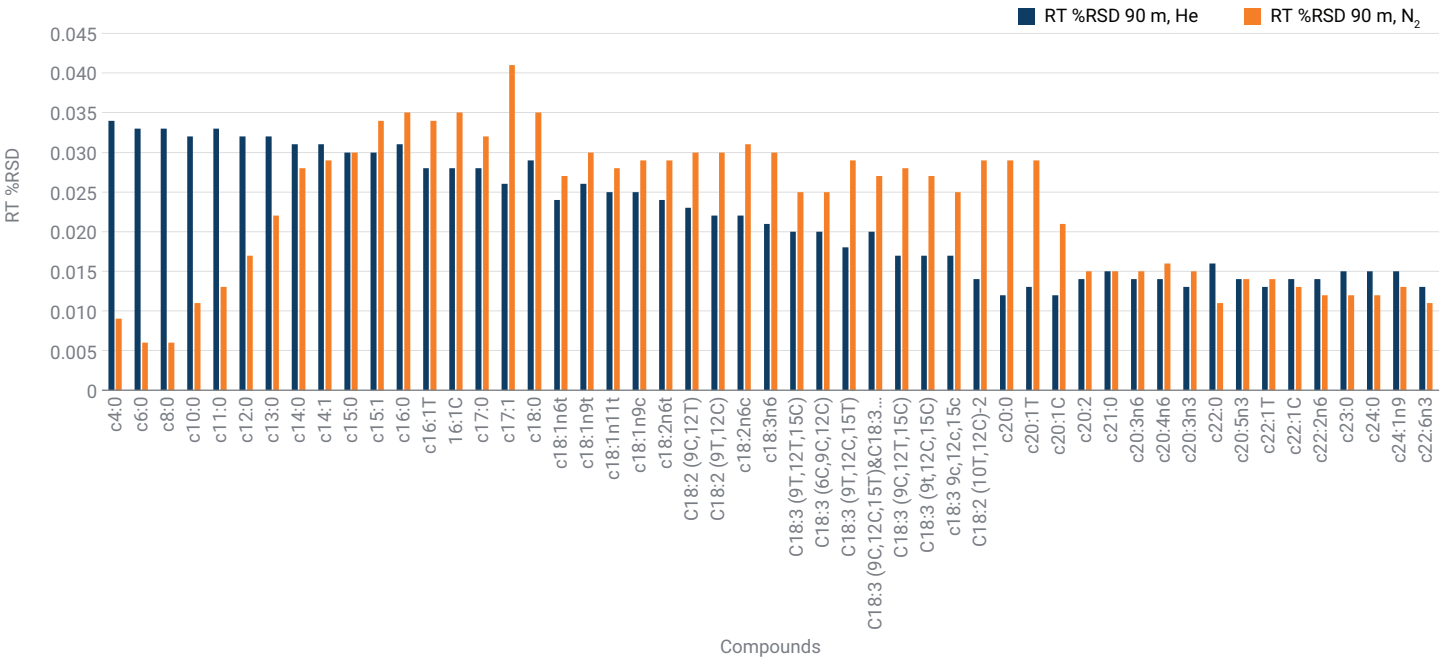

Figure 9. Retention time precision on the 90-meter Agilent J&W DB-FastFAME column.

Conclusion

A high-speed FAMEs analysis was achieved on an Agilent 8850 GC system with excellent repeatability when running the FAMEs standards on specially engineered 20-meter and 30-meter Agilent J&W DB-FastFAME columns under optimized oven programs. The analysis time is less than 10 minutes using He carrier gas and less than 15 minutes using N_2 carrier gas, which is five to eight times faster than the conventional method. Peak resolutions obtained by the fast methods met the AOAC 996.06 standard requirements. The methods can help improve lab productivity significantly for routine analysis of 37 FAMEs.

The analysis of 52 FAMEs on 90-meter DB-FastFAME and 100-meter Agilent J&W HP-88 columns demonstrated that the 8850 GC can deliver performance equivalent to the Agilent 8890 GC system when analyzing complex fatty acids—including *cis*/*trans* fatty acids and positional isomers—in terms of resolution, area repeatability (< 2.5%), and RT precision (< 0.04%).

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2. AOAC International. AOAC Official Methods of Analysis (2000), Method 996.06, Ce 2–66.
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