Application Note

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Fatty Acid Methyl Esters (FAMEs) Analysis on an Agilent 8890 GC and Its Application to Real Samples

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Abstract

This Application Note applied methods GB5009.168-2016[1] and 5009.257-2016[2] to FAMEs analysis using a long polar column in an Agilent 8890 GC. The GC method was optimized for the separation of 37 representative FAMEs and 21 representative *trans* FAMEs in 80 minutes. The system retention time (RT), area repeatability, and linearity were evaluated, and a FAMEs mixture prepared from real oil samples was analyzed.

Introduction

Fats are primarily the triesters of fatty acids and glycerol, and are commonly called triglycerides. For nutrition labeling purposes, fat is defined as the sum of the fatty acids in the food, regardless of source, expressed as triglyceride equivalents. There are different types of fatty acids, classified according to their degree of unsaturation: saturated, monounsaturated, and polyunsaturated. *Trans* fatty acids are unsaturated fatty acids that contain at least one nonconjugated and *trans* double bond.

The fat content in food has always been a widely discussed and scrutinized element of nutrition. Many shoppers are interested in the amount of fat in food for health, nutrition, weight loss, and more.

A number of methods, such as GB 5009.168-2016[1] and GB 5009.257-2016[2], have been developed for the analysis of fats in food. These two methods describe approaches for extracting fats from different food matrices, *trans*-esterification of the fatty acids into fatty acid methyl esters (FAMEs), and recommended GC methods for separation and data analysis. Method 168 mainly focuses on the analysis of 37 representative fatty acids, whereas method 257 focuses on the analysis of *trans* fatty acids.

Materials

Equipment

- Agilent 8890 GC equipped with a split/splitless inlet and FID detector
- Agilent 7693A automatic liquid sampler (ALS) (p/n G4567A)

Chemicals

- 37-component FAMEs mix (CDAA-252795-MIX-1 mL), purchased from ANPEL Laboratory Technologies (Shanghai) Inc., containing C4–C24 FAMEs in the 200–400 ng/µL concentration range.
- 13-Component *trans* FAMEs Mix (CDAA-2527,15–100 mg) and eight component *cis/trans* octadecatrienoic acid methyl esters (CDAB-CRM47792), purchased from ANPEL Laboratory Technologies (Shanghai) Inc. The weight % for each component in the two mixtures was in the range of 3–30 %.

Samples

Samples of soybean oil, peanut oil, and sesame oil were provided and prepared by the Shanghai Institute of Quality Inspection and Technical Research according to GB 5009.168-2016.

Instrumental

Table 1. Instrument conditions.

GC system	8890A GC
S/SL Inlet	250 °C , split ratio 100:1,
Liner	Split, Ultra inert, glass wool, low pressure drop (p/n 5190-2295)
Oven ramp program	100 °C (13 minutes), 10 °C/min to 180 °C (6 minutes), 1 °C/min to 200 °C (20 minutes), 4 °C/min to 230 °C (7 minutes)
Carrier gas	Nitrogen, 40 psi, constant pressure mode
Column	Agilent HP-88, 100 m × 0.25 mm, 0.20 µm (p/n 112-88A7)
Detector	280 °C, H_2: 40 mL/min Air: 400 mL/min Make up gas: 25 mL/min

Results and discussion

The oven temperature program recommended in GB 5009.168-2016 was used. Constant pressure mode was used, and column head pressure was optimized at 40 psi to give satisfactory separation with minimum resolution of 1.3 for critical pairs, that is, C20:0/C20:3n6, exceeding the resolution requirement of 1.25 specified by the method.

A mixture standard of 37 FAMEs was diluted to 50–100 ng/µL for each component, and used to test system repeatability. This standard was chosen in accordance with the GB method, and because it was designed to mimic the fatty acid composition of many food samples. The oven ramp program was quite long; as shown, the 37 FAMEs were separated in 81 minutes (Figure 1). All components were well resolved. The overlaid chromatograms from six injections showed excellent area and RT repeatability (Figure 1). Table 2 lists the RT, area, and precision of each peak. The area repeatability is in the range of 1.1–3.4 % (Figure 2), with the area RSD% of one component reaching 4.0 %. Since the sample solvent was hexane, and the run time was more than 80 minutes per injection, the evaporation of sample (especially solvent) during separation resulted in a slight variation in the sample concentration.

The sample quantity loaded onto the column for each component was in the range of 0.5–1 ng. This low amount of sample, compounded by solvent evaporation, resulted in area RSD% slightly beyond 2 %, but still in accordance with the quantitation requirement.

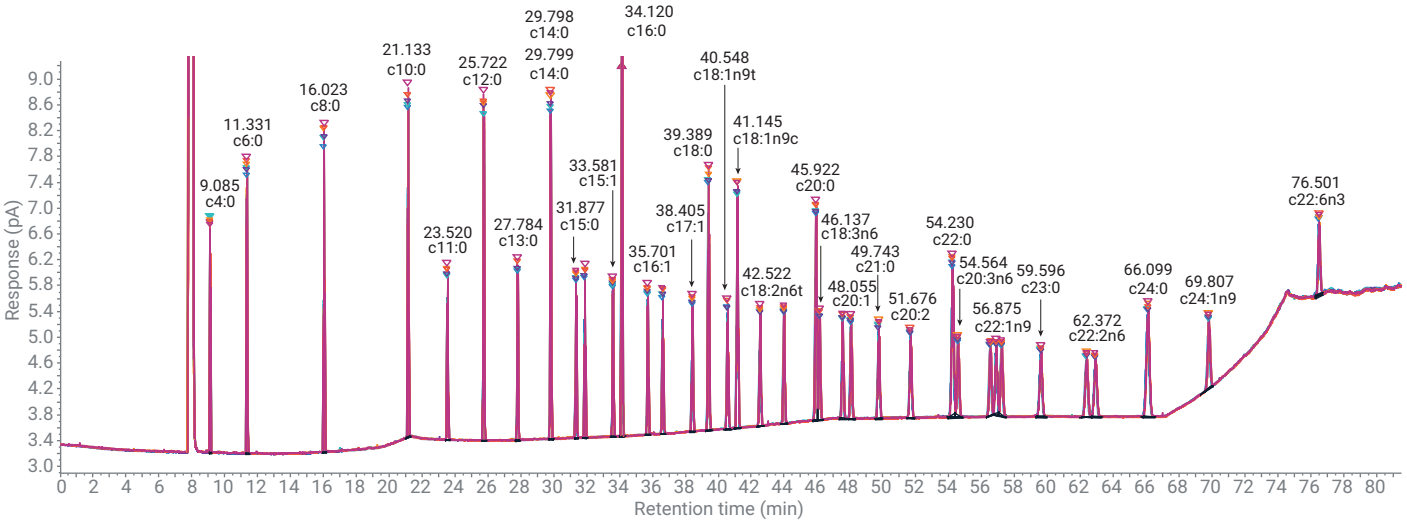

Figure 1. Overlaid chromatograms of six injections of 37 FAMEs on an 8890 GC.

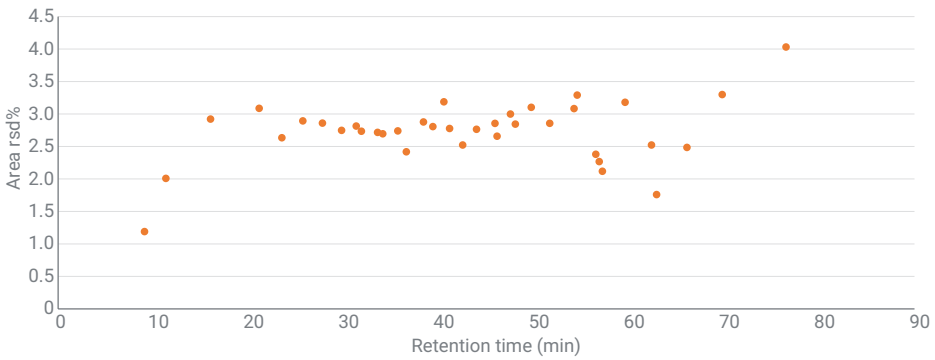

Figure 2. Area precision of 37 FAMEs in six injections.

Retention time repeatability was in the range of 0.01–0.03 % (Figure 3). Although the long run time made excellent system repeatability more difficult to achieve, the 8890A GC delivered accurate, precise, and stable control of oven temperature, inlet pressure, and detector flow rates, helping to generate highly repeatable chromatograms, and ensure reliable identification results.

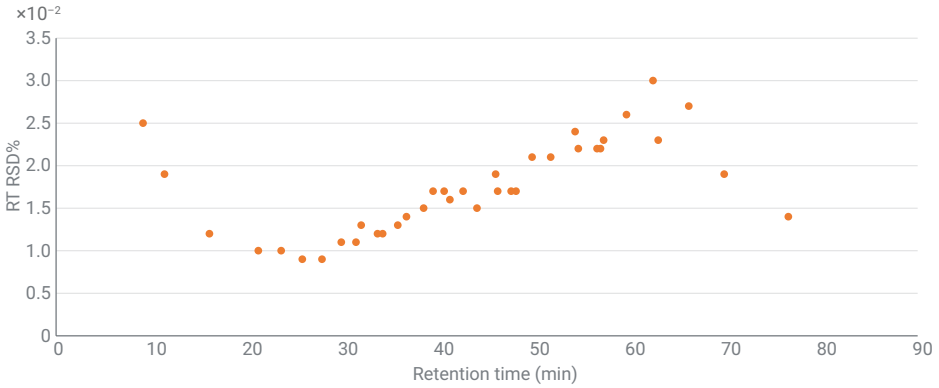

Figure 3. RT precision of 37 FAMEs in six injections.

Table 2. RT, area, and their precision for 37 compounds in six injections.

Compound	Mean RT (min)	RT RSD (%)	Area average	Area RSD (%)
C4:0	9.086	0.025	6.903	1.189
C6:0	11.331	0.019	12.795	2.008
C8:0	16.022	0.012	16.599	2.921
C10:0	21.131	0.01	17.995	3.086
C11:0	23.518	0.01	9.365	2.633
C12:0	25.721	0.009	18.702	2.893
C13:0	27.783	0.009	9.655	2.859
C14:0	29.796	0.011	19.362	2.747
C14:1	31.333	0.011	9.57	2.813
C15:0	31.874	0.013	9.9	2.733
C15:1	33.58	0.012	9.818	2.716
C16:0	34.119	0.012	29.97	2.694
C16:1	35.699	0.013	9.999	2.739
C17:0	36.602	0.014	10.125	2.417
C17:1	38.4	0.015	10.053	2.876
C18:0	39.385	0.017	20.432	2.805
C18:1n9t	40.544	0.017	10.294	3.186
C18:1n9c	41.142	0.016	20.389	2.776
C18:2n6t	42.519	0.017	10.188	2.522

Compound	Mean RT (min)	RT RSD (%)	Area average	Area RSD (%)
C18:2n6c	43.972	0.015	10.363	2.763
C20:0	45.919	0.019	20.719	2.854
C18:3n6	46.135	0.017	10.003	2.657
C18:3n3	47.54	0.017	10.167	2.998
C20:1	48.052	0.017	10.354	2.843
C21:0	49.731	0.021	10.554	3.102
C20:2	51.671	0.021	10.302	2.855
C22:0	54.225	0.024	21.046	3.082
C20:3n6	54.554	0.022	10.28	3.29
C20:3n3	56.514	0.022	10.244	2.379
C22:1n9	56.871	0.022	10.273	2.266
C20:4n6	57.204	0.023	10.633	2.117
C23:0	59.588	0.026	10.693	3.179
C22:2n6	62.36	0.03	10.415	2.521
C20:5n3	62.903	0.023	10.177	1.758
C24:0	66.093	0.027	21.326	2.484
C24:1n9	69.797	0.019	10.863	3.298
C22:6n3	76.499	0.014	9.456	4.03

System linearity was evaluated by calculating the relative standard deviation (RSD%) of response factor (RF) of C18:1c FAME and C18:2c FAME at five concentration levels. Table 3 shows RF RSD% as low as 4 % for the two probe compounds, which demonstrates excellent linearity in terms of peak response. Some labs use the ESTD method for quantitation; good detector linearity across a wide concentration range can ensure accurate quantitation even when a single-point ESTD method is used.

Figure 4 shows the separation of a mixture of 13-component *trans* FAMEs and 8-component octadecatrienoic acid methyl esters isomers. The results were achieved according to GB 5009.257-2016. The oven temperature program used was the same as for the analysis of the 37 FAMEs mixture. The eight isomers of C18:3 *trans* FAMEs isomers are particularly challenging to resolve, especially considering that other FAMEs that coexisted with C18:3 FAMEs must be resolved in the same run. However, the 100 m HP-88 column resulted in eight peaks for the eight isomers in the enlarged elution section for octadecatrienoic acid methyl esters (Figure 5). Although the resolution was far from baseline separation, comparison with the reference chromatogram in GB 5009.257-2016 shows that the separation achieved was within acceptable limits. Additionally, the cis-9,*cis*-12,*cis*-15-octadecatrienoic acid methyl ester was well separated from other seven *trans* isomers. This is an important practical consideration in light of the nature of *trans* fatty acids labeling in the nutrition labeling industry.

Table 3. Area response linearity for C18:1-*cis* and C18:2-*cis*.

Component	Concentration	Area (PA*S)	RF (Response per amount)	RSD% of RF
C18:1-*cis*	1.7 ppm	0.330	0.194	4.7 %
	17 ppm	3.002	0.177	
	170 ppm	29.152	0.171	
	1,700 ppm	301.107	0.177	
	17,000 ppm	3,065.390	0.180	
C18:2-*cis*	0.86 ppm	0.155	0.180	3.0 %
	8.6 ppm	1.628	0.189	
	86 ppm	14.833	0.172	
	860 ppm	152.562	0.177	
	8,600 ppm	1,550.921	0.180	

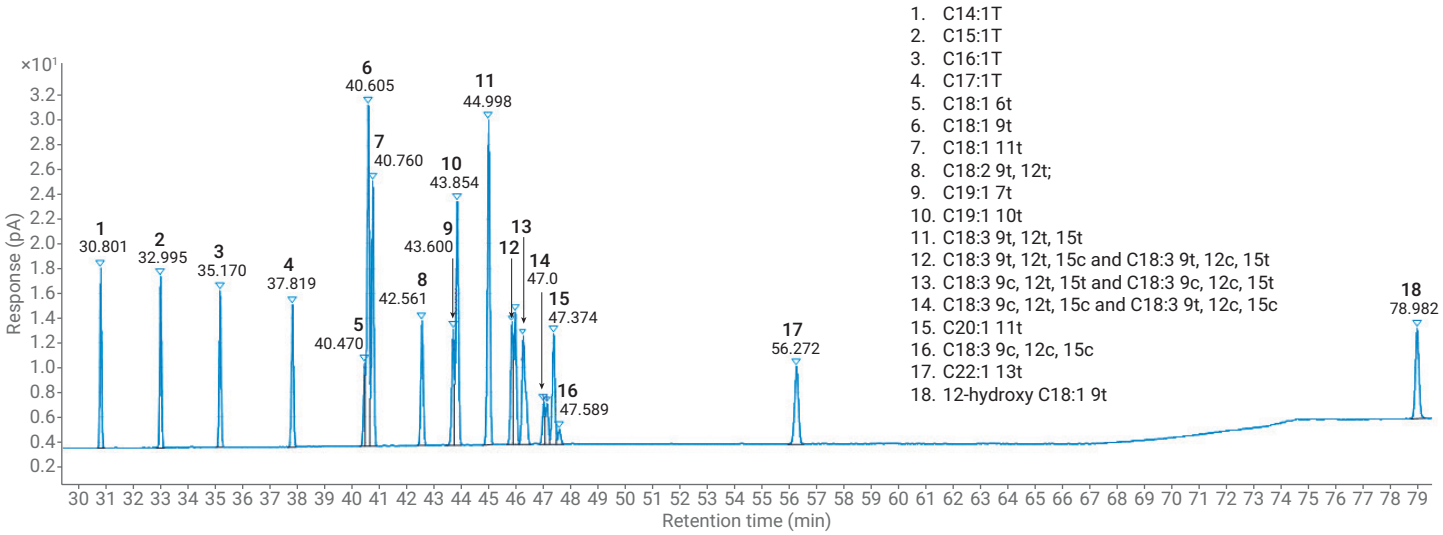

Figure 4. Chromatogram of 21 *trans* FAMEs on an HP-88 column.

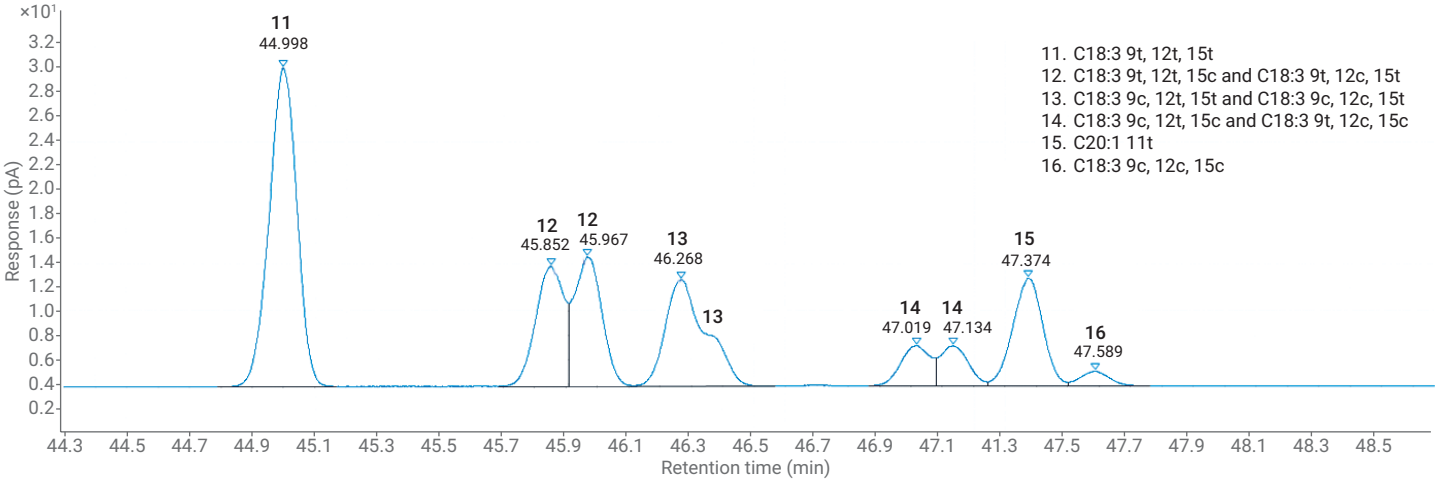

Figure 5. Enlarged chromatogram for octadecatrienoic acid methyl ester isomers.

Real oil samples, including soybean oil, peanut oil, and sesame oil, were extracted, derivatized, and analyzed on an 8890 GC platform according to GB 5009.168-2016. Figures 6A, 6B, and 6C show the resulting chromatograms. C16:0, C18:0, C18:1n9c, C18:2n6c, C18:3n3, and C20:1 were the main fatty acids identified in the three types of oil samples (The red font label in Figures 6A, 6B, and 6C are compounds listed in the method calibration table but not identified in the real sample).

There is a solution for FAMEs analysis that provides a fast analysis to resolve the 37 representative FAMEs on a short polycyanopropyl siloxane column within 10 minutes[3]. However, the fast analysis has certain limitations for the separation of *cis* and *trans* FAMEs. The 60–80 minutes FAMEs analysis using a long polar column was developed to deal with samples that require separation of complex *cis/trans* fatty acids or other challenging isomers. In certain applications, such as quality testing of extra virgin olive oil, effective separation of *cis/trans* FAMEs is more important than the analysis time.

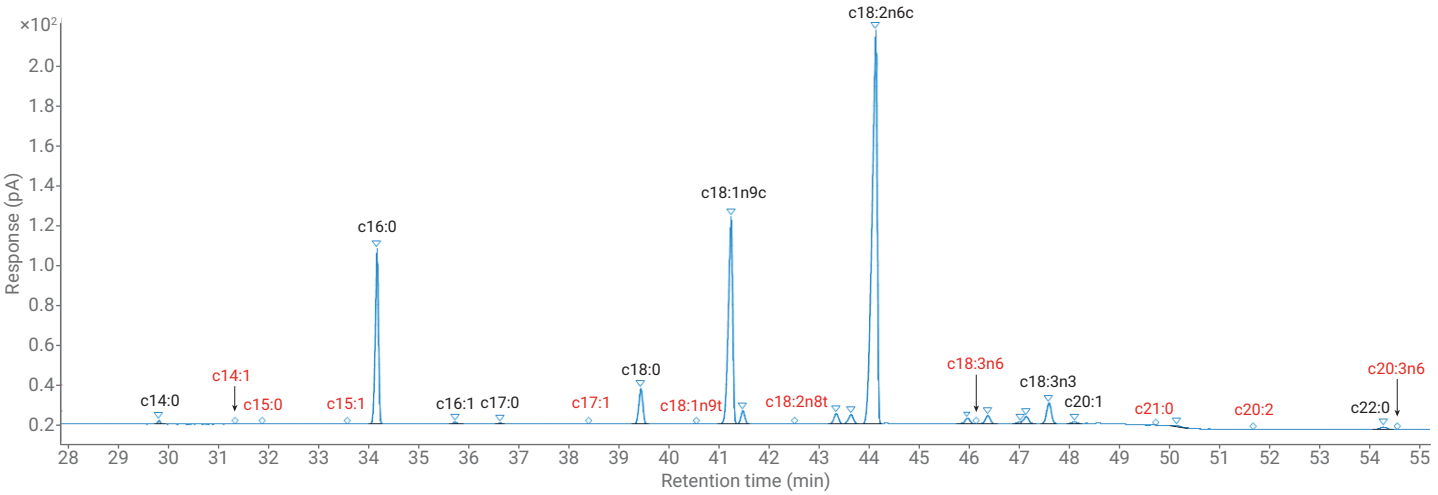

Figure 6A. Chromatogram for sesame oil analysis.

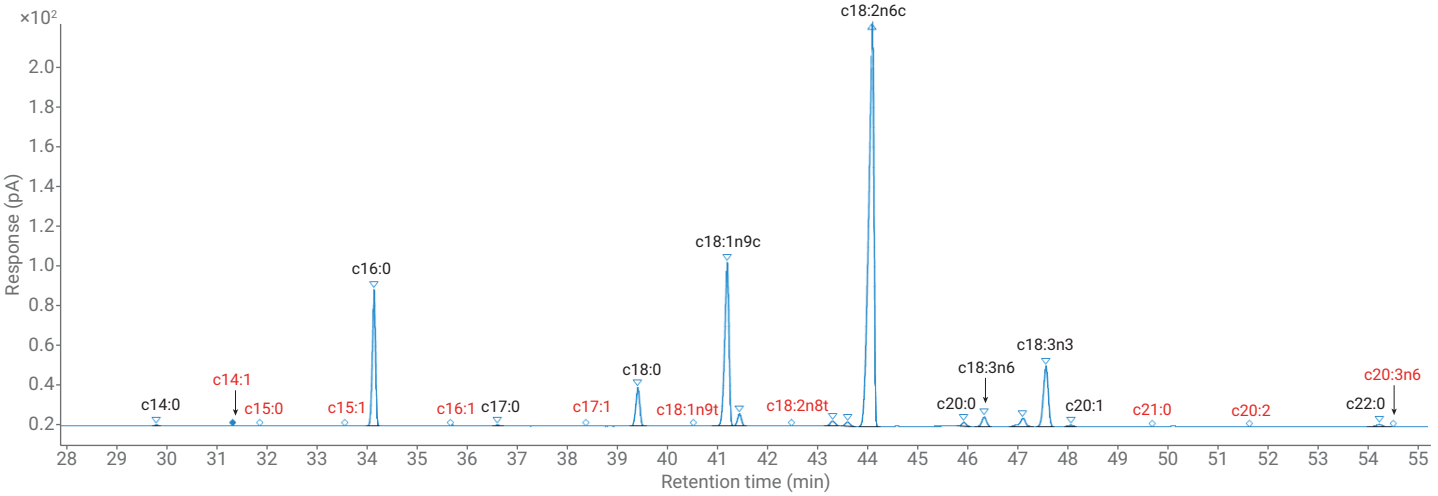

Figure 6B. Chromatogram for soybean oil analysis.

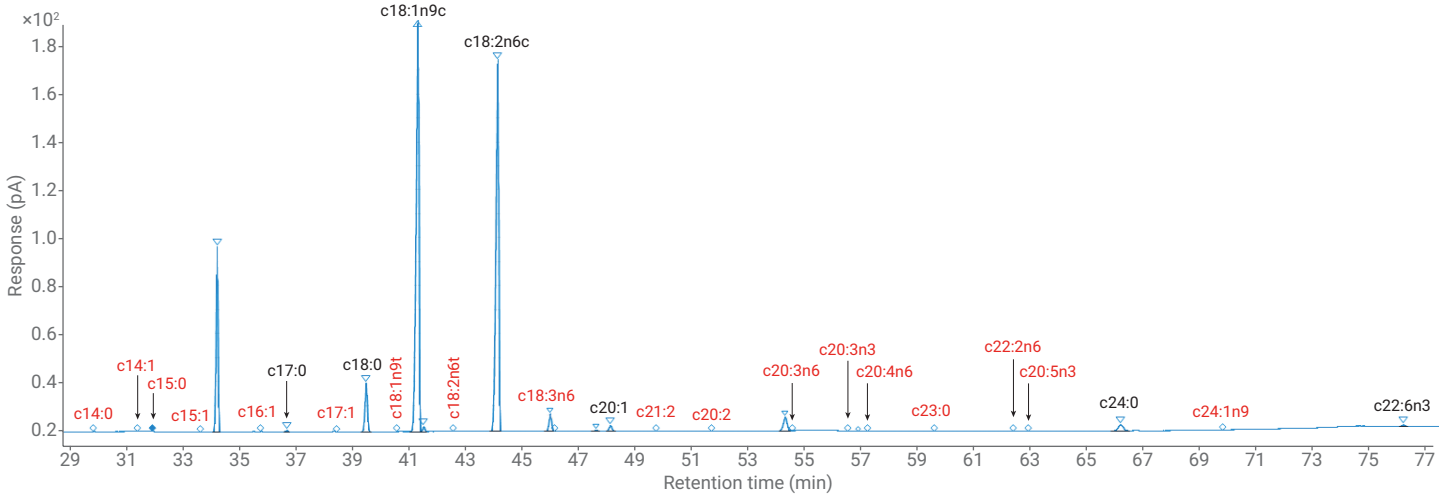

Figure 6C. Chromatogram for peanut oil analysis.

Conclusions

Use of an 8890A GC coupled with an HP-88 column for the analysis of 37 representative FAMEs and 21 *trans* FAMEs resulted in good resolution with both types of samples. Resolution of the critical compounds pair met, and exceeded, the requirements of methods GB 5009.168-2016 and GB 5009.257-2016. Excellent retention time, area repeatability, and the wide linear detection range of the FID proved that the 8890A GC is an ideal platform for the reliable analysis of FAMEs.

References

1. Determination of fatty acids in food, GB5009.168-2016 method.
2. Determination of *trans* fatty acids in food, GB5009.257-2016 method
3. A fast analysis of FAME by Intuvo 9000 GC, *Agilent Technologies Application Note*, publication number 5991-9482EN.

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