Analysis of Fatty Acids in Infant Formulas Using an Agilent J&W HP-88 Capillary GC Column

Application Note

Food Safety

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Abstract

This application note describes an efficient and economical RTL-GC/FID method for determination of fatty acids in infant formulas. The fatty acids were converted to FAMEs using acetyl chloride-methanol methyl esterification method.
Agilent J&W HP-88 capillary GC column provides excellent separation for complex FAMEs including *cis-trans* isomer separation.

Agilent Technologies

Introduction

Infant formulas are widely used as substitutes for human milk, the balance of nutrients supplied in formulas can thus have far-reaching, long-term consequences. Since fat comprises a major component of infant formula, it is especially important to consider the possible implications of the fatty acid composition of this fat, because the fatty acids play important roles in the biological systems. They are the constituents of the lipids in the biological membranes which influence membrane properties such as the fluidity, integrity, permeability, and the activities of the membrane bound enzymes [1]. Especially some long-chain polyunsaturated fatty acids such as docosahexaenoic acid (DHA), arachidonic acid (ARA) and eicosapentaenoic acid (EPA) are important for normal visual and brain development. However, it is important that *trans*-fatty acids should not be used in standard infant formulas. In the unlikely event that partially hydrogenated fats are used in formulas, trans-fatty acids should not exceed 3% of total fatty acids.

GC is by far the most widely used method for the analysis of fatty acids [2], and the fatty acids are generally analyzed as their fatty acid methyl ester (FAME). Agilent can provide different solutions for analysis of FAMEs [3] to meet different requirements.

In 2010, Chinese regulation GB/T 5413.27-2010 was issued to monitor fatty acids in infant's and children's food [4]. According to this regulation, this application note demonstrates the separation on the HP-88 for FAME column of a 37-component mixture by gas chromatography- Flame Ionization Detection (GC-FID) and GC/MSD.

Experimental

Chemicals and standards

Reference standard mixtures of FAMEs were purchased from Seperlco Co.,Ltd.(Shanghai, China). The 37-component mixture (Supelco #18919) is available as a 100-mg neat mixture, containing C4-C24 FAMEs (2%-4% relative concentration). Standard solution was diluted in 10 mL hexane before use. Final concentration of each FAME was 0.2-0.4 mg/mL.

Sample preparation

Weigh, to the nearest 0.1 mg, approximately 500 mg of sample in a 20-mL screw-cap tube. Dissolve the sample in 5 mL of toluene; add 6 mL of 10% acetyl chloride-methanol solution in the tube. Close the tube, and incubate in a water bath at 80 °C for 2 hours, then cool to room temperature. Transfer the solution into a 50-mL centrifuge tube; wash the tube with 6% Na_2CO_3 solution. Combine all of the Na_2CO_3 solution into the 50-mL centrifuge tube, centrifuge at 5,000 rpm for 5 minutes. Transfer the clear supernatant into a sample vial for subsequent GC analysis.

Instrumentation

The experiment was performed on an Agilent 7890A GC-FID system and an Agilent 7890 GC-5975C MSD system. The instrumental conditions are listed Tables 1 and 2.

Table 1. Instrumentation and Analytical Conditions for the GC/FID System

GC chromatograph	Agilent 7890A Series
Autosampler	Agilent 7683 Injector and sample tray
Column	HP-88, 100 m × 0.25 mm × 0.2 µm (p/n 112-88A7)
GC Inlet	260 °C, split ratio 30:1
Carrier gas	Nitrogen, constant flow mode, 1 mL/min
Retention time locking	C16:0 locked to 18.600 min
Oven temperature program	140 °C (5 min), 4 °C/min to 240 °C (15 min)
Detector	FID @ 280 °C
Inlet liner	Split liner, tapered deactivated (p/n 5183-4647)
Injection volume	1 µL

Table 2. Instrumentation and Analytical Conditions for the GC/MS system

GC chromatograph	Agilent 7890A Series
Autosampler	Agilent 7683 Injector and sample tray
Column	Agilent J&W HP-88, 100 m × 0.25 mm × 0.2 µm (p/n 112-88A7)
GC Inlet	260 °C, split ratio 30:1
Carrier gas	Helium, constant flow mode, 20 cm/s
Oven temperature program	140 °C (5 min), 4 °C/min to 240 °C (15 min)
Injection volume	1 µL
Mass selective detector	5975C MSD
Transfer line	280 °C
Solvent delay	8.2 min
Acquisition mode	Scan (40-400 amu)

Results and Discussion

The described GC/FID method is used for quality control of FAMEs. A typical chromatogram for the analysis of the 37-component FAMEs reference standard, obtained on the HP-88 column is shown in Figure 1. As indicated in the chromatogram, most of target compounds can be baseline separated by an Agilent J&W HP-88 GC column with excellent peak shapes, except for the following compounds: C20:3n3, C22:1n9, and C20:4n6 (ARA). These compounds also can not be baseline separated in GB/T 5413.27-2010. ARA is an omega-6 polyunsaturated fatty acid [C20:4 (n-6)], naturally present in human mother's milk. ARA is commonly added to infant formula products as an important nutrient. Resolution of ARA and C22:1n9 is about 1 in Figure 1, and this resolution can be better with a higher split ratio. This separation of these compounds is sufficient for infant and children food analysis. Compared to the chromatogram in Chinese Regulation GB 5413.27-2010, elution order of C18:3n3, C22:1n9, and C20:5n3 (EPA) are different in Figure 1; because different

cyanopropyl-polysiloxane column can provide different retention time of FAMEs.

Standard solution was analyzed by GC/MS to reduce the risk of incorrectly identifying FAMEs. The total ion chromatograms (TICs) obtained for the 37-component FAMEs reference standard is given in Figure 2. The same separation was achieved as GC-FID. This GC-MS method is also useful for the determination of FAMEs in other complex mixture.

Table 3. FAMEs, Retention Time, CAS Number, Molecular Form and Molecular Weight

Peak #	RT(min)	Compound	CAS No.	Mol form	MW
1	8.82	Butyric acid methyl ester (C4:0)	623-42-7	C5H10O2	102
2	9.23	Caproic acid methyl ester (C6:0)	106-70-7	C7H14O2	130
3	9.96	Caprylic acid methyl ester (C8:0)	111-11-5	C9H18O2	158
4	11.20	Capric acid methyl ester (C10:0)	110-42-9	C11H22O2	186
5	12.06	Undecanoic acid methyl ester (C11:0)	1731-86-8	C12H24O2	200
6	13.09	Lauric acid methyl ester (C12:0)	111-82-0	C13H26O2	214
7	14.29	Tridecanoic acid methyl ester (C13:0)	1731-88-0	C14H28O2	228
8	15.63	Myristic acid methyl ester (C14:0)	124-10-7	C15H30O2	242
9	16.75	Methyl myristoleate (C14:1)	56219-06-8	C15H28O2	240
10	17.07	Pentadecanoic acid methyl ester (C15:0)	7132-64-1	C16H32O2	256
11	18.27	*cis*-10-Pentadecenoic acid methyl ester (C15:1)	90176-52-6	C16H30O2	254
12	18.60	Palmitic acid methyl ester (C16:0)	112-39-0	C17H34O2	270
13	19.64	Palmitoleic acid methyl ester (C16:1)	1120-25-8	C17H32O2	268
14	20.16	Heptadecanoic acid methyl ester (17:0)	1731-92-6	C18H36O2	284
15	21.23	*cis*-10-Heptadecenoic acid methyl ester (C17:1)	75190-82-8	C18H34O2	282
16	21.74	Stearic acid methyl ester (C18:0)	112-61-8	C19H38O2	298
17	22.37	*trans*-9-octadecenoic methyl ester (C18:1n9t)	2462-84-2	C19H36O2	296
18	22.69	Oleic acid methyl ester (C18:1n9c)	112-62-9	C19H36O2	296
19	23.35	Linolelaidic acid methyl ester (C18:2n6t)	2566-97-4	C19H34O2	294
20	24.08	Linoleic acid methyl ester (C18:2n6c)	112-63-0	C19H34O2	294
21	24.87	Arachidic acid methyl ester (C20:0)	1120-28-1	C21H42O2	326
22	25.07	γ-Linolenic acid methyl ester (C18:3n6)	16326-32-2	C19H32O2	292
23	25.68	Linolenic acid methyl ester (C18:3n3)	301-00-8	C19H32O2	292
24	25.83	*cis*- 11-Eicosenoic acid, methyl ester (20:1)	2390-09-2	C21H40O2	324
25	26.43	Heneicosanoic acid methyl ester (C21:0)	6064-90-0	C22H44O2	340
26	27.29	*cis*- 11,14-Eicosadienoic acid methyl ester (C20:2)	2463-02-7	C21H38O2	322
27	28.05	Behenic acid methyl ester (C22:0)	929-77-1	C23H46O2	354
28	28.38	*cis*- 8,11,14-Eicosatrienoic acid, methyl ester (C20:3n6)	21061-10-9	C21H36O2	320
29	29.04	*cis*- 11,14,17-Eicosatrienoic acid methyl ester (C20:3n3)	55682-88-7	C21H36O2	320
30	29.11	Erucic acid methyl ester (C22:1n9)	1120-34-9	C23H44O2	352
31	29.22	Arachidonic acid methyl ester (C20:4n6)	2566-89-4	C21H34O2	318
32	29.71	Tricosanoic acid methyl ester (C23:0)	2433-97-8	C24H48O2	368
33	30.74	*cis*-13,16-Docasadienoic acid, methyl ester (C22:2n6)	61012-47-3	C23H42O2	350
34	31.16	*cis*- 5,8,11,14,17-Eicosapentaenoic acid methyl ester (C20:5n3)	2734-47-6	C21H32O2	316
35	31.48	Lignoceric acid methyl ester (C24:0)	2442-49-1	C25H50O2	382
36	32.74	Nervonic acid methyl ester (C24:1n9)	2733-88-2	C25H48O2	380
37	36.54	*cis*- 4,7,10,13,16,19-Docosahexaenoic acid methyl ester (C22:6n3)	301-01-9	C23H34O2	342

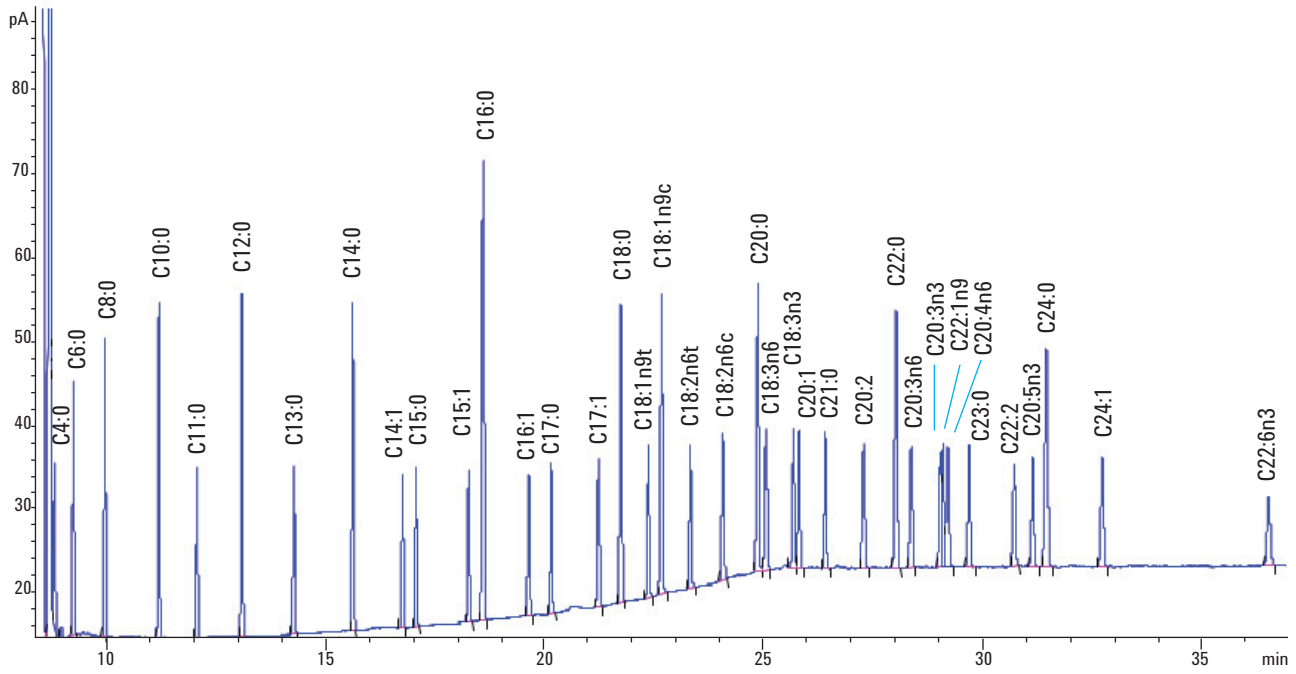

Figure1. GC-FID analysis of 37-component FAMEs standard mixture on Agilent J&W HP-88, 100m × 0.25 mm × 0.20 µm column. (GC-FID method see Table 1).

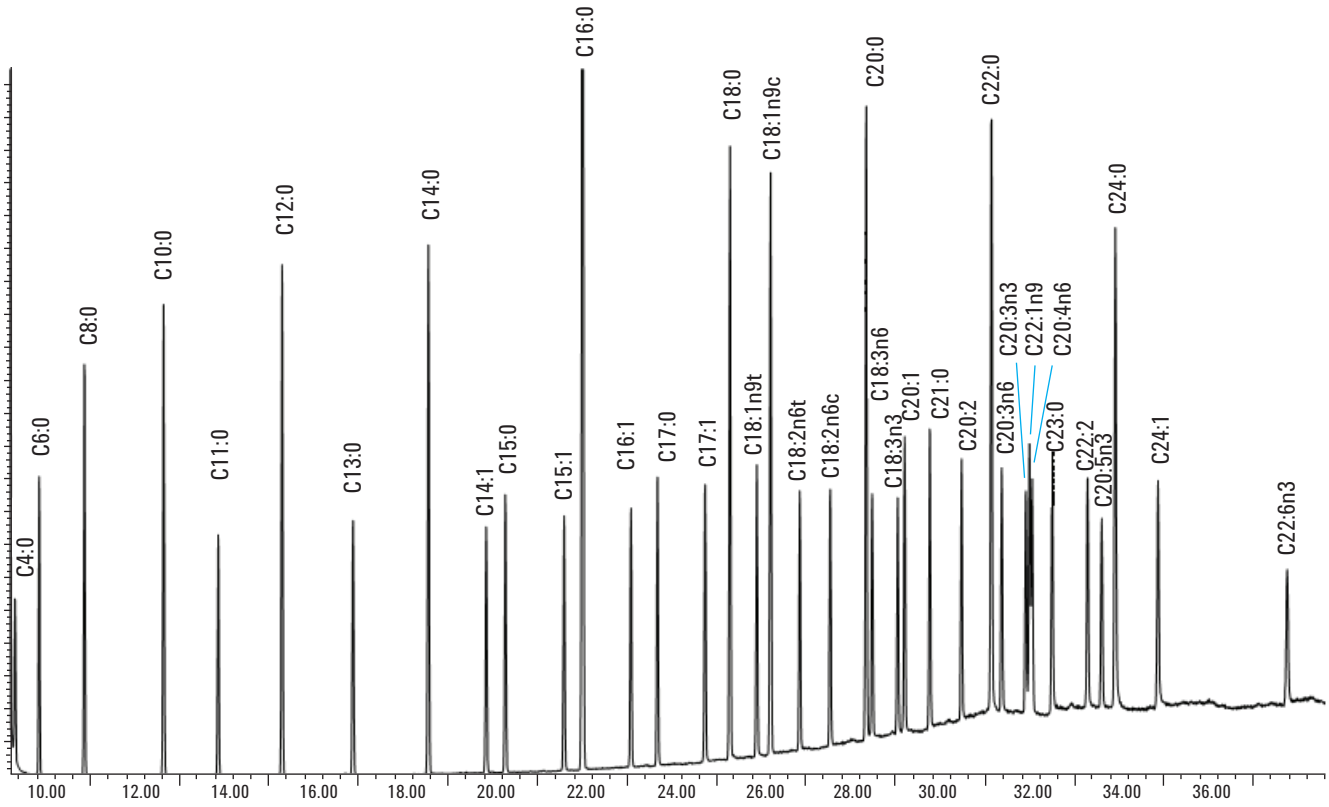

Figure 2. GC-MS analysis of 37-component FAMEs standard mixture on Agilent J&W HP-88, 100 m × 0.25 mm × 0.20 µm column. (GC-MS method see Table 2).

Real sample

All test samples were treated according to the procedure described in the sample preparation. One example of infant formula analysis is shown in Figure 3. When analyzing real samples, there are significant differences in the actual composition of fatty acids in milk samples. Concentration ranges from 0.01 to 5%. Therefore, retention time of each compound may change. Standard solution should be prepared according to the concentration of real samples.

Retention Time Locking (RTL) is a good tool to reproduce retention times on any Agilent GC instrument [5]. The GC/FID system was retention time locked (RTL) to C16:0 at 18.60 min. Retention time of each compound is listed in Table 3.

Figure 4 demonstrates chromatograms of another real sample extract (milk powder) and standard mixture using RTL-GC-FID method. It shows excellent reproducibility. Using this method for quality control of fatty acids in infant formula, excellent separation was obtained, satisfying regulatory requirements.

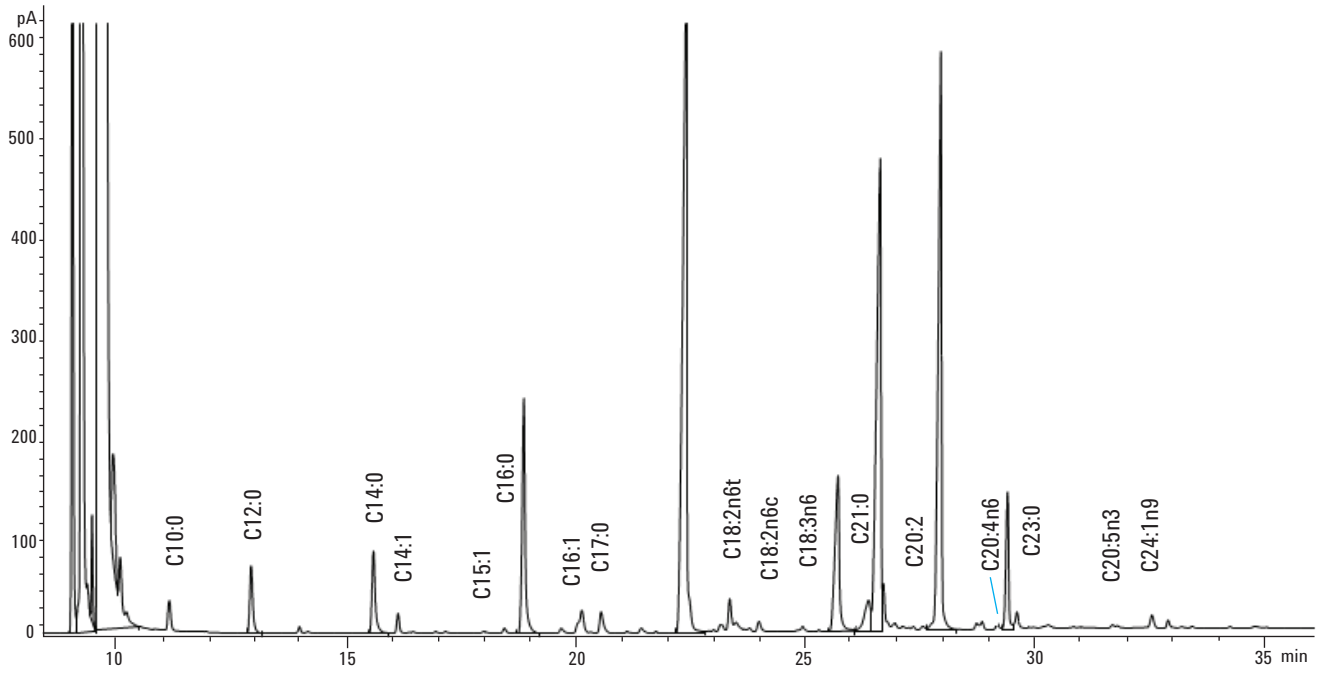

Figure 3. GC-FID analysis of FAMEs from infant formula on an Agilent J&W HP-88 GC 100 m × 0.25 mm × 0.20 µm column.

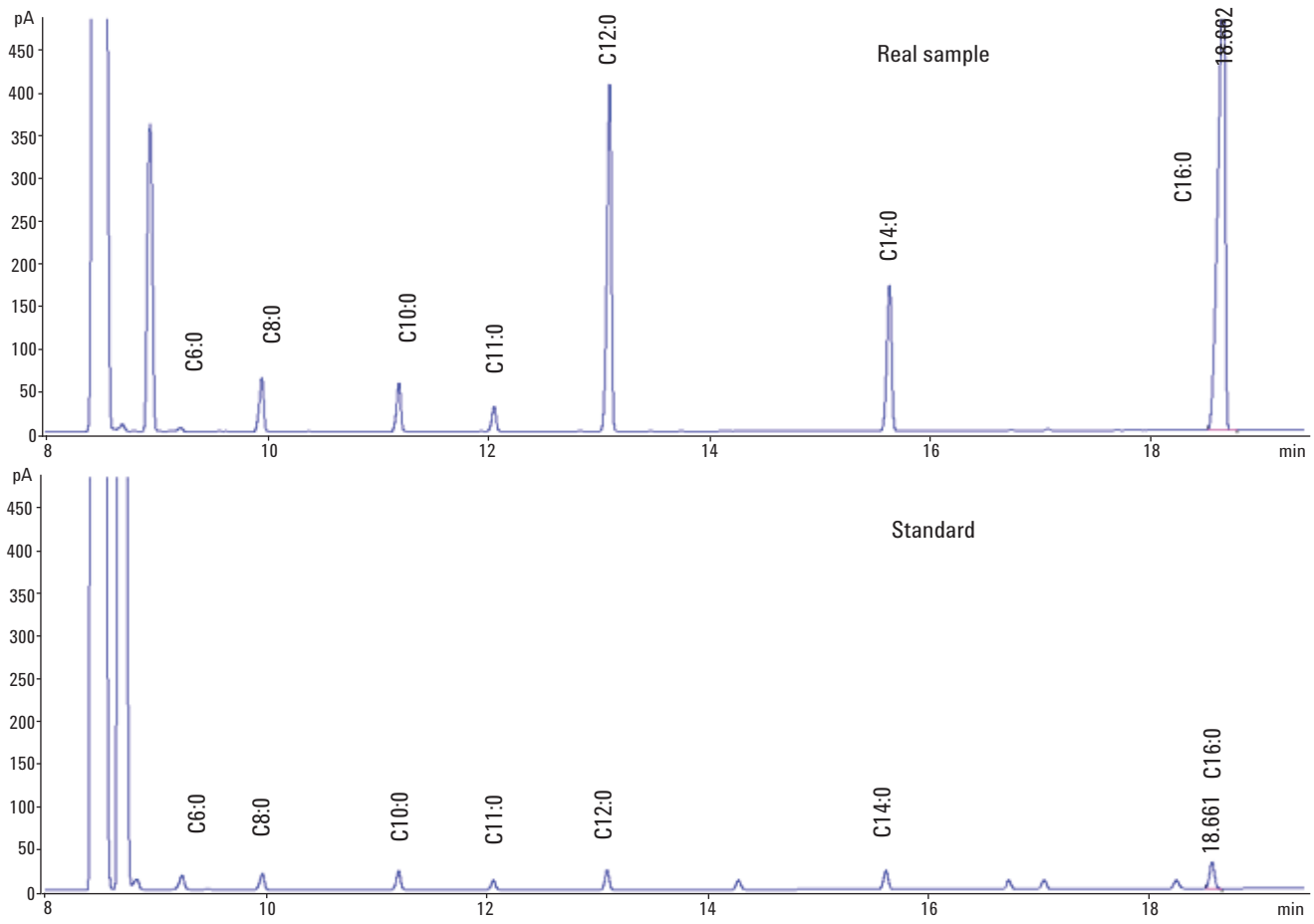

Figure 3. GC/FID chromatograms of another real sample extract (milk powder) and standard mixture using Agilent J&W HP-88, 100 m × 0.25 mm × 0.20 µm column.

Conclusion

This application note demonstrates an efficient and economical RTL-GC/FID method for determination of fatty acids in infant formulas. GC/FID and GC/MS were used to analyze 37-component FAME standards. The Agilent J&W HP-88 GC column can effectively separate the FAMEs with excellent peak shape. It can deliver reliable results while meeting the requirements of regulatory methods.

References

1. Stubbs CD, Smith AD. The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. Biochim Biophys Acta. 1984 Jan 27; 779(1):89-137."

2. Eder K. Gas chromatographic analysis of fatty acid methyl esters. J Chromatogr B Biomed Appl. 1995 Sep 15; 671(1-2):113-131.

3. Frank David, Pat Sandra and Allen K. Vickers "Column selection for the Analysis of Fatty Acid Methyl Esters", Agilent Technologies, publication 5989-3760EN, www.agilent.com/chem.

4. National food safety standard (China), GB 5413.27-2010: Determination of fatty acids in foods for infants and young children, milk and milk products.

5. V.Giarocco, B. Quimby, and M. Klee, "Retention Time Locking: Concepts and Applications," Agilent Technologies, publication 5966-2469EN, www.agilent.com/chem.

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