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

Application Note

Food Safety

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.

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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% Na2CO3 solution. Combine all of the Na2CO3 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.

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.

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<th>MW</th>
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Figure 1. GC-FID analysis of 37-component FAMEs standard mixture on Agilent J&W HP-88, 100 m × 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.
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


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.


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