Detection of trans fatty acids in milk powder

Abstract: Method provided in the national food safety standard GB 5413.36—2010 "Determination of trans fatty acids in foods & dairy products for infants and young children" was referred in this experiment, to determinate content of trans fatty acid in milk powder.

In the experiment, the fat in the milk powder were extracted with organic solvent, then the extracted fat were methyl-esterified under alkaline conditions to form methyl esters of fatty acids. The fatty acid methyl esters formed were analyzed by a Gas Chromatograph equipped with a hydrogen flame ionization detector (FID) and quantified by external standard method.

Key words: Trans-9 methyl octadecanoate, trans-9, 12 methyl octadecadienoate, external standard method, fat

Trans fatty acids, also known as trans fats, can be divided into two categories: first one is natural type, which is present in beef and mutton and their milk; the other one is artificially manufactured type, which is produced in the processing and cooking of animal and vegetable lipids. Long-term, excessive consumption of trans-fatty acids can be severely harmful to human health.

“Reducing the intake of foods containing trans-fatty acids” is a healthy lifestyle that the China Ministry of Health has been actively promoting. The content of trans fatty acids in infant foods and dairy products is low, but long-term consumption would still interfere with the utilization of essential fatty acids in infants and young children, and cause central nervous system developmental disorders. Therefore, the regulation, monitoring and detection of trans fatty acids in foods are necessary.

1 Experiment

1.1 Reagents and standard solutions

Petroleum ether: boiling range 30 °C ~ 60 °C.

Ethyl ether: Analytical grade

95% ethanol: Analytical grade

Hexane: Chromatography grade

Ammonia: 25 %～28 %, Analytical grade

KOH: Analytical grade

Methanol: Analytical grade

Anhydrous sodium sulfate (Na_2SO_4): Analytical grade

FAME standard: Trans-9 methyl octadecanoate (C18:1-9t), Trans-9, 12 methyl octadecadienoate (C18:2-9t, 12t); purchased from BEHRINGER Reagent Co., Ltd.

4 mol/L KOH-CH_3OH: 26.4g of KOH was weighed, dissolved with CH_3OH and about 5g Na_2SO_4 was added to make the solution volume up to 100 ml. Solution filtered & stored.

Preparation of the standard solutions:

Standard stock solution of trans FAME: Concentration is 10.0 mg/ mL respectively. 100 mg (precision down to 0.1 mg) of trans-9 methyl octadecanoate standard and trans-9, 12 methyl octadecadienoate standard were respectively weighed, dissolved in n-hexane and made their volume up to 10.0 mL; stored in the refrigerator below -15 °C.

Standard intermediate solution of trans FAME: Concentration is 1.0 mg/ mL respectively. Respectively 5.0 mL of two standard FAME stock solutions were pipetted into same 50 mL volumetric flask, diluted with n-hexane and made up to its nominal volume. Prepared before use. This would serve as the highest concentration of the standard curve.

Standard working solution of trans FAME: Prepared before use. Respectively 0, 2.0, 6.0, 8.0, 10.0 mL of trans FAME standard intermediate solution were pipetted into respective 10 mL volumetric flasks, and made up to their nominal volume with n-hexane. The concentration of these standard working solutions would then be 0, 0.2, 0.6, 0.8, 1.0 mg/mL.

1.2 Instruments and working conditions

Thermostatic water bath：40 ℃～80 ℃

Centrifuge：speed≥4000 rpm

Analytical balance： precision of 0.1 mg

Vortex mixer

Rotary evaporator

Liposuction tube：

GC4000A（EAST & WEST ANALYTICAL INSTRUMENTS, INC.）

GC instrumental conditions：

Column：HP-88 100m×0.25mm×0.20μm； Vaporization chamber temperature：250℃； FID temperature：300℃； Carrier gas：High-purity N_2; Split ratio：70:1；

Flow rate in column：1mL/min； Injection volume：1μL; Oven temperature:

Temperature ramping conditions:

Heating rate（℃/min）	Temperature (℃)	Holding time（min）
	120	0
10	175	10
5	210	5
5	230	5

Table 1 GC Temperature Ramp conditions

1.3 Sample treatment

1.5 g (precision down to 0.1 mg) well mixed solid sample is weighed and put into the liposuction tube, 10 mL of water at 45 °C ±2 °C was added to rinse and mix the sample up, until the sample completely dispersed, and then cooled to room temperature.

Fat extraction: 3.0 mL ammonia was added to the liposuction bottle, mixed well, placed in a

water bath at 60 °C ± 2 °C for 15 min to 20 min, cooled to room temperature. 10 mL of ethanol and 1 drop of Congo red solution were added and mixed well. 25 mL ethyl ether was added, capped, and shaken for 1 min. Then 25 mL of petroleum ether was added, shaken for 1 min, centrifuged at 4000 rpm for 10 min. The supernatant was decanted into a round bottom flask (for a rotary evaporator) as the first extraction. 5 mL ethanol, 25 mL ether and 25 mL petroleum ether were added to the remaining sample solution for second extraction according to the above procedure. After centrifugation, the supernatant was decanted and combined with the first supernatant. The round bottom flask was placed on a rotary evaporator, and the solvent was removed by rotary evaporation under nitrogen at 60 °C ± 2 °C, and the residue was retained, which is fat.

Preparation of FAME: The above fat was dissolved in n-hexane and made up to 10.0 mL. 3.0 mL was taken into a 10 mL test tube, and 0.3 mL of potassium hydroxide-methanol solution was added, capped tightly, shaken vigorously on a vortex shaker for 2 min, centrifuged at 4000 rpm for 5 min, and the supernatant was transferred to a gas chromatograph vial, as the sample solution.

2 Results & Discussion

2.1 Standard curve

The concentrations of the experimentally prepared trans fatty acid standard working solution are as below:

Table 2 Concentrations of the standard working solution

Name	Concentration mg/mL				
trans-9 methyl octadecanoate（C18:1-9t）	0	0.1814	0.5442	0.7256	0.9070
trans-9, 12 methyl octadecadienoate（C18:2-9t, 12t）	0	0.1694	0.5082	0.6776	0.8470

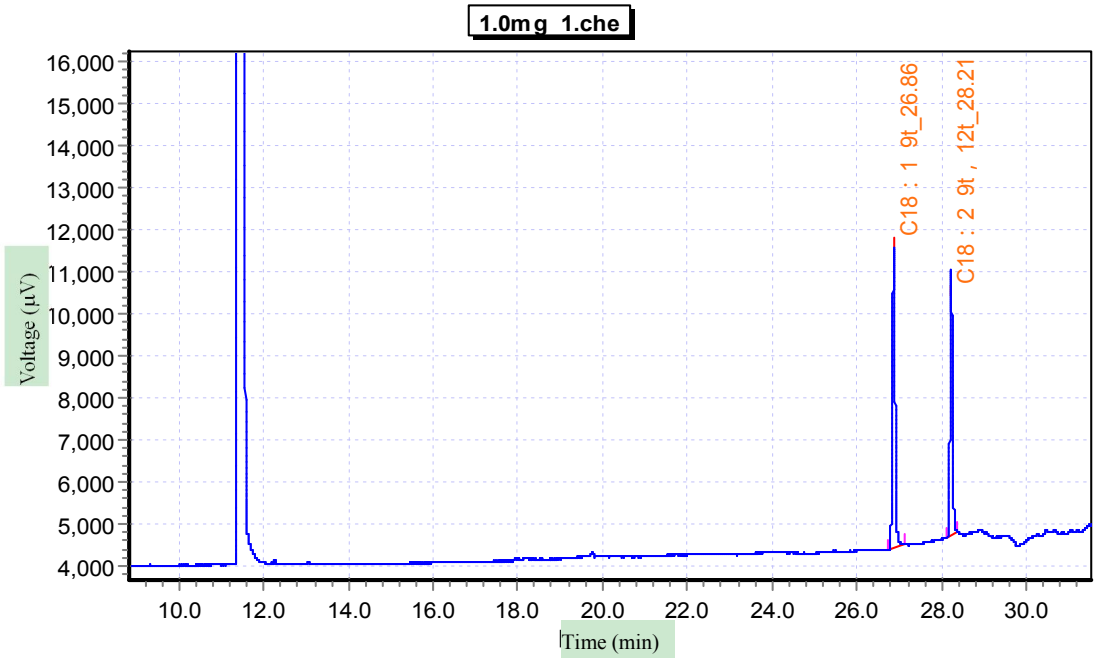

Figure 1 Chromatogram of the standard solution

Under the instrumental conditions mentioned above, the standard curves of the two trans FAME were plotted based on their concentration and peak area, as shown in Fig. 1 to Fig. 3. Among them, Figure 1 is the separation chromatogram of the standard solution. As the trans-9 methyl octadecanoate obtained in experiment was at 0~0.9mg/mL, and the trans-9, 12 methyl octadecadienoate at 0~0.85mg/mL, the concentration and peak area are in good linear relationships and their correlation coefficients are 0.9991 and 0.9995, respectively.

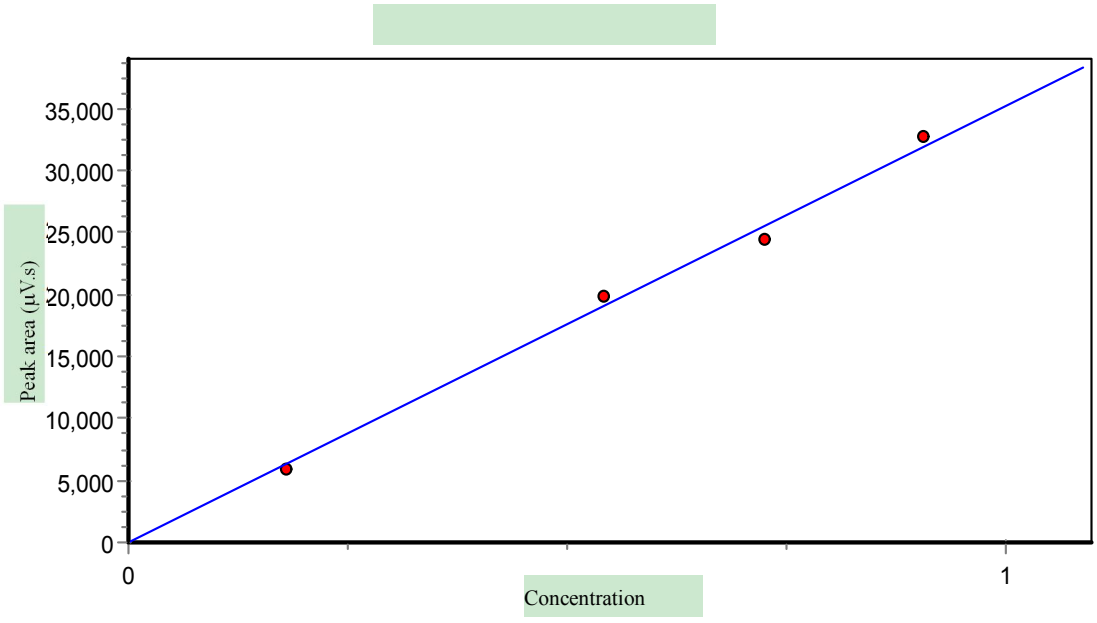

Figure 2 Calibration curve of the trans-9 methyl octadecanoate

calibration curve： Y = 35223.966102X +0.0000

correlation coefficient： r = 0.9991

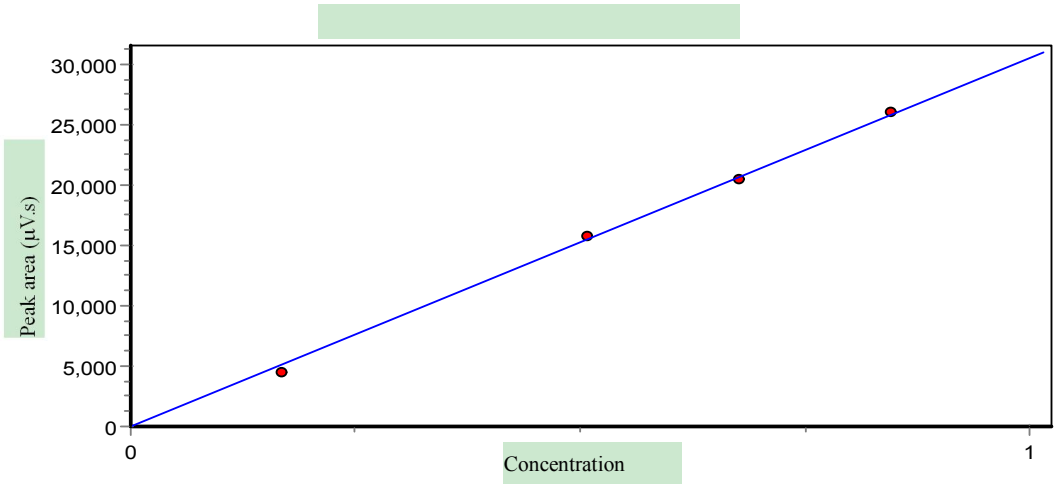

Figure 3 Calibration curve of the trans-9, 12 methyl octadecadienoate

calibration curve：Y = 30457.65138 X +0.0000

correlation coefficient：r = 0.9995

2.2 Sample analysis results

Two milk powder samples were separately subjected to trans fatty acid extraction, to methyl esterification and then to GC analysis, according to the experimental methods mentioned above. Based on the peak area of trans-9 methyl octadecanoate and trans-9,12 methyl octadecadienoate in the milk powder sample, quantitative analysis were carried out by external calibration method according to the respective calibration curves, to obtain their concentration in different samples.

The results are shown in Table 3. Fig. 4 and Fig. 5 are chromatograms of the 1# sample and the 2# sample, respectively.

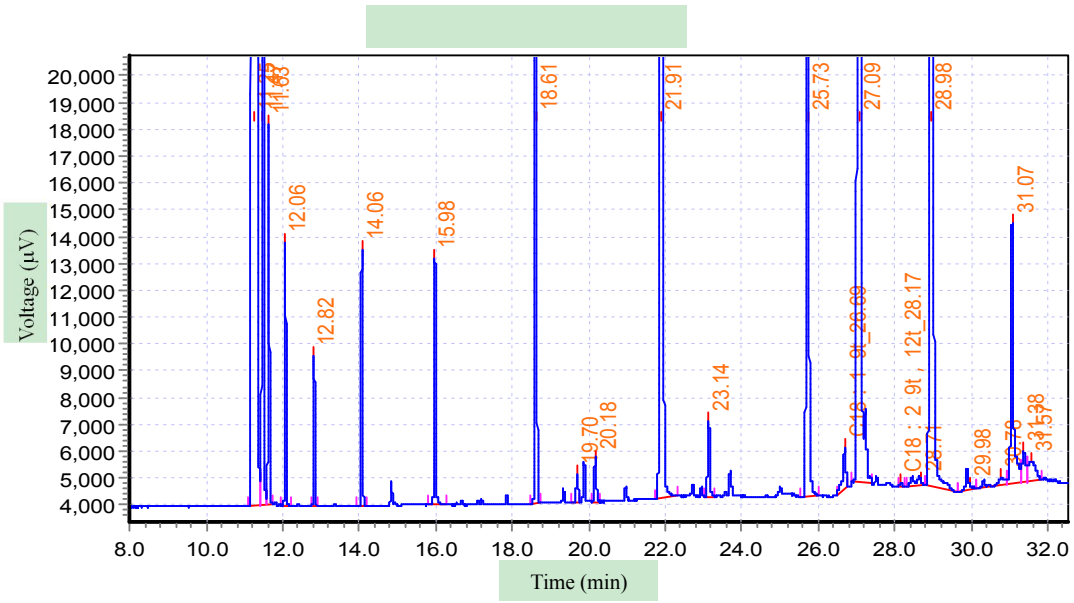

Figure 4 Chromatogram of sample 1

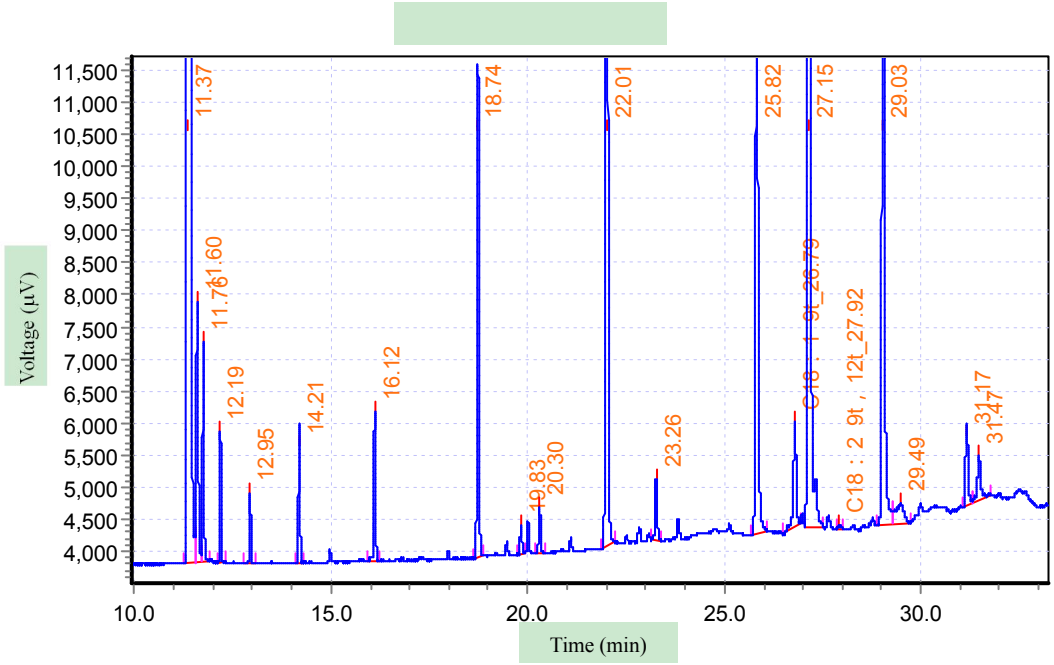

Figure 5 Chromatogram of sample 2

Table 3 Mass concentration of trans FAME in sample extract

Name	concentration mg/mL	
	C18: 1-9t	C18: 2-9t, 12t
Sample 1#	0.2198	0.02
Sample 2#	0.2263	0.01

The result obtained above is the mass concentration of trans FAME in the sample extract, and as this result put into Equation 1, the content of trans-9 octadecanoic acid and trans-9, 12 octadecadienoic acid in the samples can be calculated. The total content of trans fatty acids in the sample is then calculated according to Equation 2. The calculation results are shown in Table 4.

$$X_{(1或2)} = \frac{c_i \times V \times M_{ai}}{m \times M_{bi}} \times 100 \qquad \textit{Equation 1}$$

X_i — content of trans octadecanoic acid or trans octadecadienoic acid in sample, in unit mg/100g;

V — volume of the sample, in unit mL;

m — sample mass, in unit g;

c_i — mass concentration of trans-9 methyl octadecanoate or trans-9, 12 methyl octadecadienoate in sample solution, in unit mg/mL;

M_{ai} — molecular mass of trans octadecanoic acid (282.46) or trans octadecadienoic acid (280.45);

M_{bi} — molecular mass of trans-9 methyl octadecenoate (296.49) or trans-9, 12 methyl octadecadienoate (294.47);

The total content of trans fatty acids in the sample, X, calculated according to equation below:

$$X = X_1 + X_2 \qquad \textit{Equation 2}$$

X — total content of trans fatty acid, in unit mg/100g；

X_1 — content of trans octadecanoic acid in sample，in unit mg/100g；

X_2 — content of trans octadecadienoic acid in sample, in unit mg/100g;

Table 4 Content of trans fatty acids in samples

Sample	X_1 (mg/100g)	X_2 (mg/100g)	X (mg/100g)
Sample 1#	138.89	12.66	151.55
Sample 2#	143.59	6.34	149.93

2.3 Precision experiment

Two independent measurements were performed under repetitive conditions, and the precision was calculated based on the measurement results. The calculation results are shown in Table 5.

Table 5 Precision experimental results

Sample		X_1	X_2
Sample 1#	1st	136.62	12.28
	2nd	141.16	13.04
Precision		3.27%	6.0%

3. Conclusion

In this paper, the content of trans fatty acids in milk powder was determined by gas chromatography with quartz capillary column, via external standard method. Those two trans fatty acids have a good linear range in their respective concentration ranges, with correlation coefficient $r \geqslant 0.999$. The instruments and methods have good repeatability and stability. The analysis result of those two types of milk powder samples show that the precision between the results obtained by the two independent experiments was less than 10%, which fully meet the requirements of international standard.