Detection of six types of fatty acids in milk powder

Abstract: Method as described in the national food safety standard GB 5413.27-2010 “Determination of fatty acids in foods and dairy products for infants and young children” was referred to by our laboratory, to determine the content of 6 types of fatty acids in milk powder. These fatty acids are lauric acid, myristic acid, γ-linolenic acid, α-linolenic acid, erucic acid and arachidonic acid (ARA).

In the experiment, the fat in the milk powder was extracted with organic solvent, then the extracted fat was saponified to form free fatty acid, and lastly, the methyl esterification reaction was carried out under the catalysis of boron trifluoride. Those fatty acid methyl ester formed were analyzed by a Gas Chromatograph equipped with a hydrogen flame ionization detector (FID) and quantified by external standard method.

Key words: fatty acid, saponification, boron trifluoride, external standard method

1 Experiment

1.1 Reagents and standard solutions preparation

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

Ether: Analytical grade

99.8% absolute ethanol: GR

Hexane: GC grade

Ammonia: 25 %～28 %, Analytical grade

KOH: Analytical grade

Methanol: HPLC grade

Boron trifluoride methanol solution: mass fraction is 14%.

Saturated NaCl solution: 18 g of NaCl dissolved in 50 mL water, stir to dissolve, clarify.

0.5 mol/L KOH-CH_3OH: 2.8 g of KOH weighed, dissolved and diluted with CH_3OH, the volume of the solution is made up to 100 ml, mixed well.

Pyrogalllic acid methanol solution (10%): 1.0 g of pyrogallic acid dissolved in 10 mL of methanol, to make a 10% solution of pyrogalllic acid methanol.

Fatty acid methyl ester standard: methyl laurate, methyl myristate, methyl γ-linolenate, methyl α-linolenate, methyl erucate and methyl arachidonate, all purchased from Behringer Reagent Co. Ltd..

Preparation of the standard solutions: the standard solutions were appropriately prepared according to the type and to the concentration of each fatty acid in the sample. N-hexane was added to make up to the volume, and then store in refrigerator at below -10 °C, valid for three months. In the experiment, the fatty acid methyl ester standard stock solution and the working solution were prepared according to the concentrations shown in Table 1.

Table 1 Concentration of fatty acid methyl ester standard solution

FAME	Concentration of the stock solution mg/mL	Concentration of the working solution mg/mL		
		1	2	3
methyl laurate	10	0.4	0.8	1.2
methyl myristate	20	1.0	1.5	2.0
Methyl γ-linolenate	1.0	0.02	0.06	0.08
Methyl α-linolenate	10	0.2	0.4	0.8
methyl erucate	1.0	0.02	0.06	0.08
methyl arachidonate	1.0	0.02	0.06	0.08

1.2 Instruments and working conditions

Thermostatic water bath：40 ℃～80 ℃

Centrifuge：speed≥5000 rpm

Analytical balance： precision 0.1 mg

Rotary evaporator

Liposuction tube：100 mL grinding test tube with stopper, dry, constant weight

GC4000A（EAST & WEST ANALYTICAL INSTRUMENTS, INC.）

GC instrument conditions：

Column：PC-2560 100m×0.25mm×0.20μm； Vaporization chamber temperature：260℃； FID temperature：280℃； Carrier gas：High-purity N_2 ； Split ratio：60:1； Flow rate in column ：1mL/min； Injection volume：1μL； Oven temperature：Initial temperature 140℃，keep for 5min, and then heat up to 240℃ at 4℃/min，keep for 30min.

1.3 Sample treatment

1.0 g (precision down to 0.1 mg) sample was weighed and put into the liposuction tube, 10 mL water at 65 °C ±1 °C was added to dissolve the sample, shaken to completely disperse the sample. 2 mL ammonia was added to the sample mentioned above, the tube was then placed in a water-bath at 65℃ ± 1℃ for 15 min, taken off and shaken gently, and then cooled to room temperature.

Fat extraction: 10 mL ethanol was added to the prepared sample, mixed well. 25 mL ethyl ether was added in, capped and shaken for 1 min. 25 mL petroleum ether was then added, capped and shaken for 1 min, and then left to separate into layers. The organic layer is transferred into the grinding flask. Then 25 mL ethyl ether and 25 mL petroleum ether were added, shaken for 1 min, and left to separate into layers. The organic layer is transferred into the grinding flask, and repeat the operation once again. The extracts are putted in a ground flask, and concentrated to dryness using a rotary evaporator.

Saponification and esterification: 1.0 mL pyrogalllic acid methanol solution (10%) is added to the concentrate. After concentration and drying, 10 mL KOH methanol solution is added and refluxed on water-bath at 80 ℃ ± 1℃ for 5 min to 10 min. 5 mL boron trifluoride methanol solution is added, continue to be refluxed for 15 min, cooled down to room temperature. The liquid is transferred into a 50 mL centrifuge tube, and the flask was washed three times with 3 mL of saturated NaCl solution respectively, and these NaCl solutions was put into 50ml centrifuge tube. 10 mL n-hexane was added, shaken and the solution was centrifuged at 5000 rpm for 5 min. The supernatant is taken as a test solution for gas chromatography analysis.

2 Results & Discussion

2.1 Standard curve

The concentration of the trans fatty acid standard working solutions prepared at the end of the experiment are as follows:

Table 2　Concentration of the standard working solution

FAME	Concentration of the standard working solution mg/mL		
	1	2	3
methyl laurate	0.5264	1.0528	1.5792
methyl myristate	1.0878	1.5062	2.092
methylγ-linolenate	0.0292	0.0876	0.1168
methylα-linolenate	0.212	0.424	0.848
methyl erucate	0.0246	0.0738	0.0984
methyl arachidonate	0.0248	0.0744	0.0992

Under the instrumental conditions mentioned above, respective standard curve for six fatty acid methyl esters were drawn based on the concentration and peak area, as shown in Figures 1 to 7. Among them, Figure 1 is the separation chromatogram of the standard solution.　Those six fatty acid methyl esters obtained in the experiment have good linear relationship between the concentration and the peak area in their respective working solution concentration range, and their correlation coefficients are ≥0.9992 respectively.

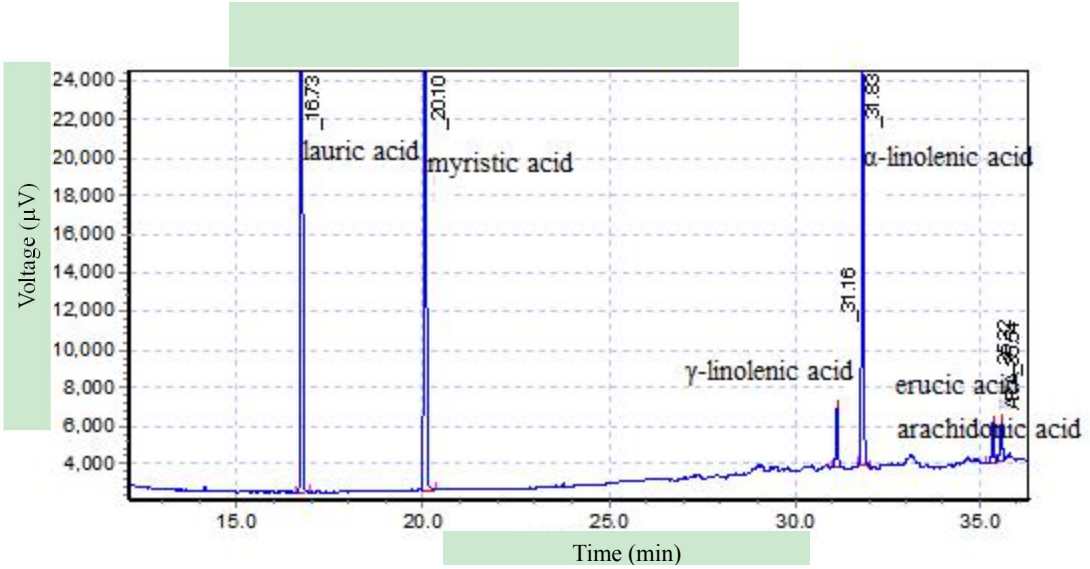

Figure 1　Chromatogram of the standard solution

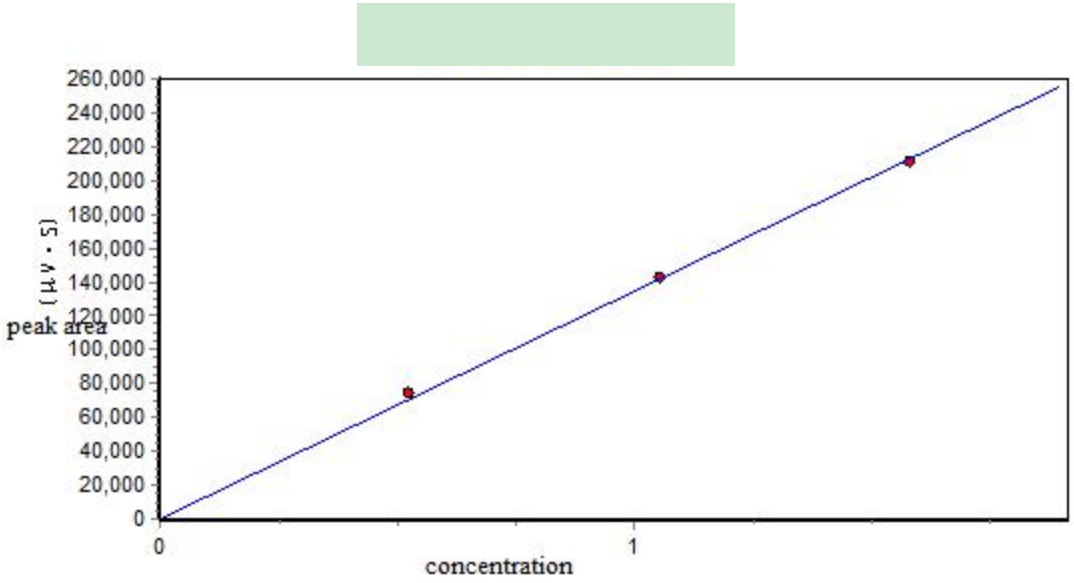

Figure 2 Calibration curve of the lauric acid

calibration curve:Y = 134960.061152 X +0.0000

correlation coefficient: 0.9997

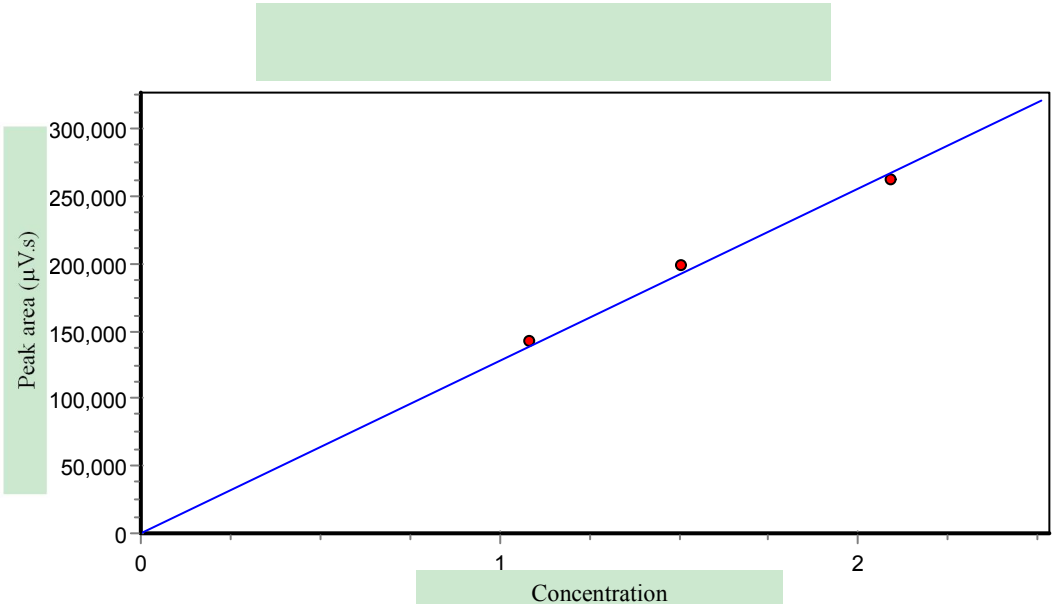

Figure 3 Calibration curve of the myristic acid

calibration curve:Y = 127750.180003 X +0.0000

correlation coefficient : 0.9992

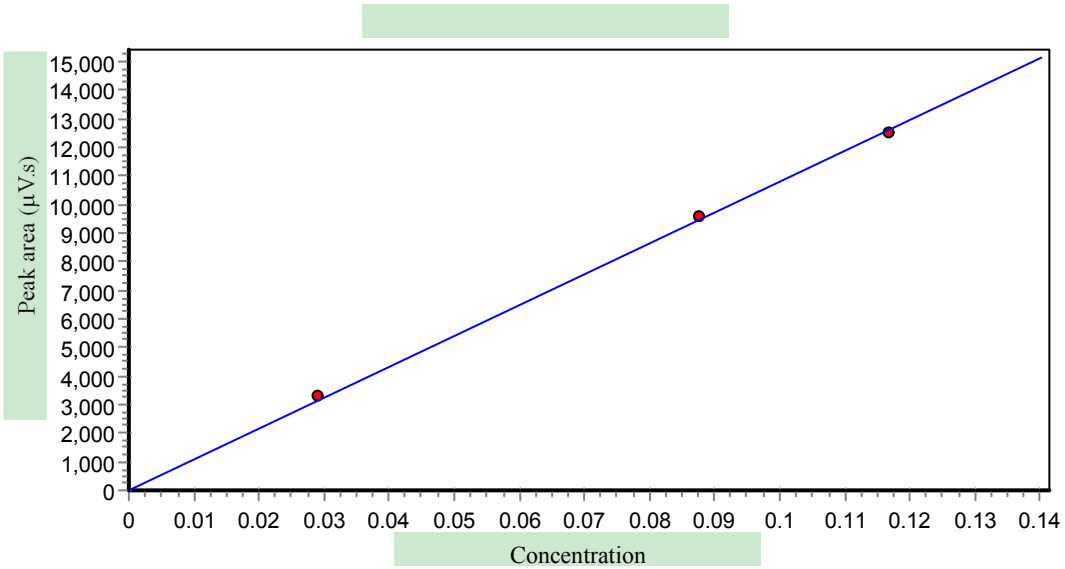

Figure 4 Calibration curve of γ-linolenic acid

calibration curve:Y = 107931.594661 X +0.0000

correlation coefficient: 0.9998

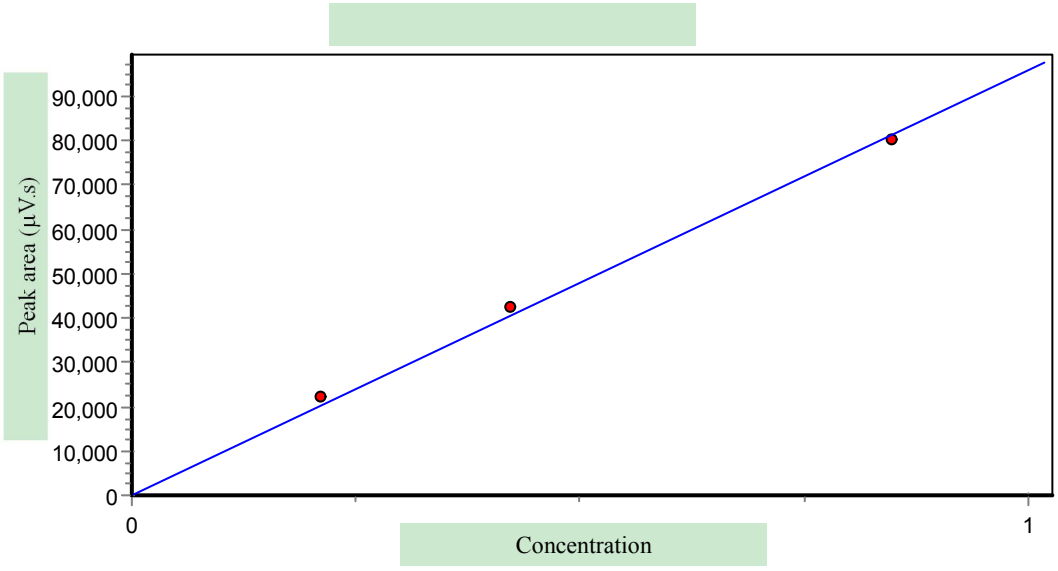

Figure 5 Calibration curve of α-linolenic acid

calibration curve:Y = 95870.460842 X +0.0000

correlation coefficient : 0.9993

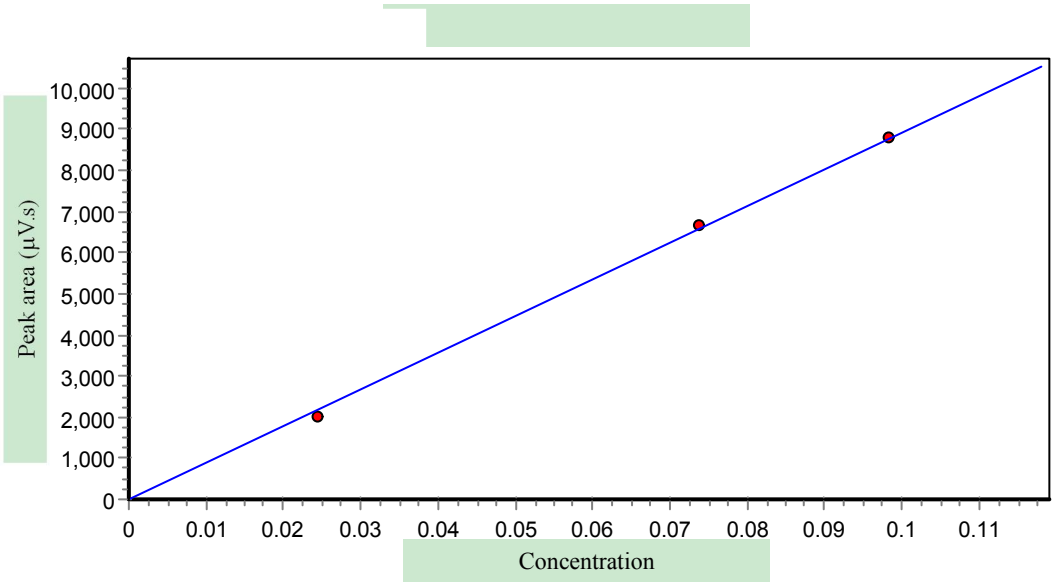

Figure 6 Calibration curve of the erucic acid

calibration curve:Y = 89144.257336 X +0.0000

correlation coefficient: 0.9996

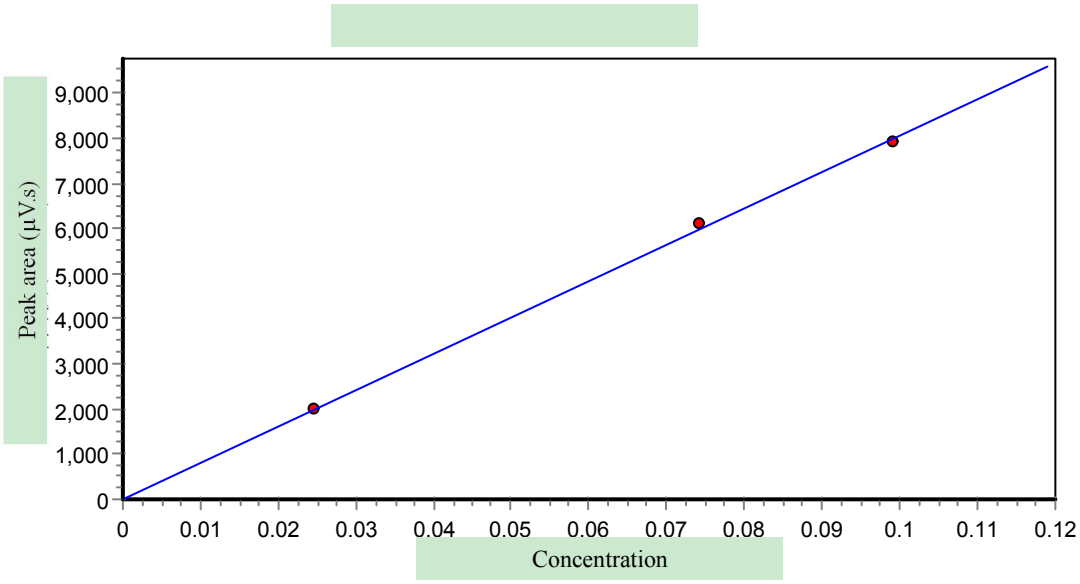

Figure 7 Calibration curve of the arachidonic acid (ARA)

calibration curve:Y = 80563.999173 X +0.0000

correlation coefficient: 0.9999

2.2 Sample analysis results

Two milk powder samples were separately subjected to fatty acid extraction and methyl esterification according to the experimental method mentioned above, and then subjected to GC analysis.

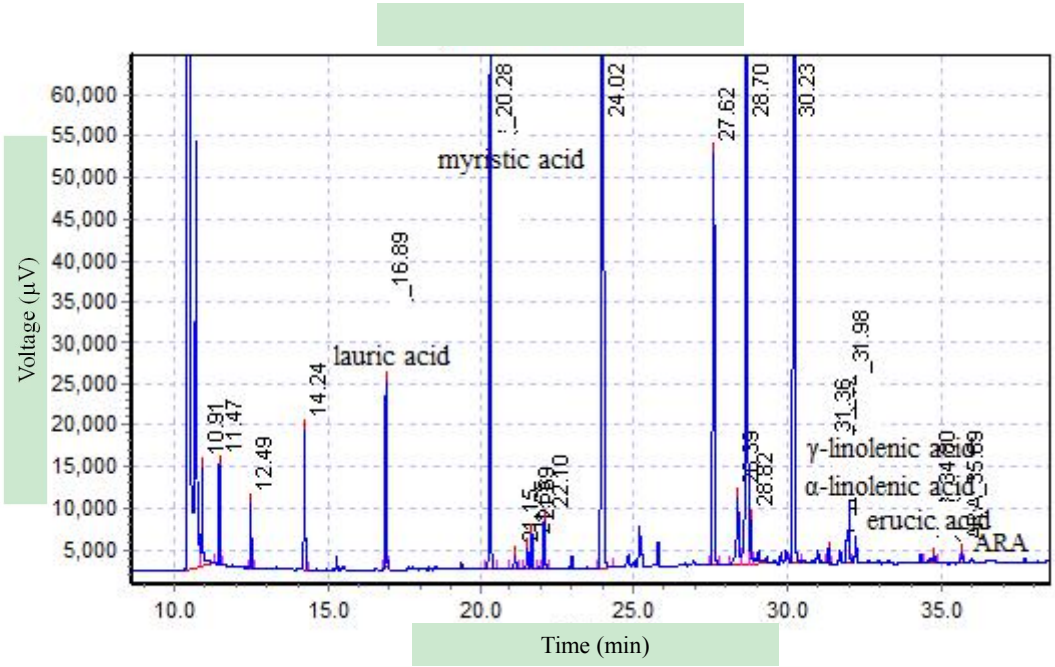

Figure 8 Chromatogram of the sample

According to the peak area of each of the six fatty acid methyl esters in the milk powder sample, combined with the respective calibration curves, the external standard method was used for quantitative analysis to obtain the concentration of six fatty acid methyl esters in different sample extracts.

The results are shown in Table 3. Figure 8 is the chromatogram of the 1# sample.

Table 3 Mass concentration of six fatty acid methyl esters in sample extracts

FAME	concentration mg/mL	
	1#sample	2#sample
methyl laurate	0.5795	0.6002
methyl myristate	1.824	1.894
methylγ-linolenate	0.0753	0.0441
methylα-linolenate	0.5298	0.3319
methyl erucate	0.00021	0.00061
methyl arachidonate	0.0768	0.0930

The result obtained above is the mass concentration of the six fatty acid methyl esters in the sample extract, and according to this result, the specific content of the six fatty acids in the sample can be calculated by combining the Equation 1. The calculation results are shown in Table 4.

$$X_{(1或2)} = \frac{c_i \times V \times F_i}{m} \times 100$$

Equation 1

X_i —— content of each fatty acid in the sample, in units mg/100g;

V —— volume of the sample, in units mL;

m —— sample mass, in unit g;

c_i —— mass concentration of each FAME in the sample solution, in unit mg/mL;

F_i —— conversion factor for conversion of each fatty FAME to fatty acid, see Attachment 1.

Table 4 Content of six fatty acids in the sample

FAME	concentration mg/100g	
	1#sample	2#sample
lauric acid	533.86	553.97
myristic acid	1680.34	1784.41
γ-linolenic acid	69.37	40.73
α-linolenic acid	488.10	306.30
erucic acid	0.20	0.56
arachidonic acid (ARA)	70.72	85.87

Attachment 1 Coefficient of conversion of each FAME to fatty acid F_i

FAME	F_i
methyl laurate	0.9346
methyl myristate	0.9421
methyl γ-linolenate	0.9520
methyl α-linolenate	0.9520
methyl erucate	0.9602
methyl arachidonate	0.9560

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

FAME	Concentration mg/100g		Precision
	1#	2#	
methyl laurate	541.71	566.63	4.5%
methyl myristate	1635.34	1734.48	5.9%
methyl γ-linolenate	67.67	71.62	5.7%
methyl α-linolenate	470.1	508.17	7.8%
methyl erucate	0.1900	0.2114	10.7%
methyl arachidonate	67.72	73.80	8.6%

3 Conclusion

In this paper, the content of six fatty acids in milk powder was determined by gas chromatography with quartz capillary column, via external standard method. In the experiment, the six fatty acid methyl esters have a good linear relationship in their respective concentration range, and the correlation coefficient is $r \geqslant 0.9992$. The instrument and method have good repeatability and stability. Two types of milk powder samples were tested and analyzed, and the corresponding contents of six fatty acids in the two milk powder samples were obtained, and the precision between the results obtained by the two independent experiments was less than 15%, which meet the requirements of the national standard.