National Standard of the People's Republic of China

GB 5413.27 – 2010

National food safety standard

Determination of fatty acids in foods for infants and young children, milk and milk products

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Preface

This standard replaces GB/T 21676 - 2008 Determination of Fatty Acids in Formula Foods and Milk Powder for Infants and Young Children, GB/T 5413.27 – 1997 Determination of DHA and EPA in Formula Foods and Milk Powder for Infants and Young Children and GB/T 5413.4 - 1997 Determination of Linoleic Acid in Formula Foods and Milk Powder for Infants and Young Children.

Comparing with the original standards, the following main changes have been made to the Standard:

- the first method is Acetyl Chloride - Methanol Esterification;
- Integrate GB/T 21676 - 2008, GB/T 5413.27 – 1997 and GB/T 5413.4 – 1997 to the second method of this standard, Ammonia Water - Ethanol Extraction Method.

Appendix A of this standard is informative.

The versions replaced by this standard are:

- GB/T 5413.4 – 1997, GB/T 5413.27 - 1997;

- GB/T 21676 - 2008.

National food safety standard

Determination of fatty acids in foods for infants and young children, milk and milk products

1 Scope

This standard provides the determination of fatty acids in infant foods and dairy.

This standard applies to determination of fatty acids in infant foods and dairy; the second method doesn't apply to determination of embedded fatty acid.

2 Normative References

The documents referenced are essential for this standard application. For dated reference documents, only the version of the noted date is suitable for this standard. For undated reference documents, the latest version (including all the revised versions) applies to this standard.

The 1st Method: acetyl chloride - methanol esterification

3 Principles

The hydrochloric acid – methanol acquired through reaction between acetyl chloride and methanol cause methyl esterification of the fats and free fatty acids in the test sample; after extracted with toluene, separate and detect the products with gas chromatograph and quantitate with the external standard method.

4 Reagents and materials

Unless otherwise specified, all reagents used are analytical reagents or above; and the water is 1st grade water specified in GB/T 6682.

4.1 Anhydrous sodium carbonate

4.2 Toluene: chromatographic pure.

4.3 Acetyl Chloride

4.4 Methanol solution of acetyl chloride (10%, v/v)

Transfer 40 mL methanol to a 100mL dry beaker, accurately pipette 5.0 mL acetyl chloride (4.3) and slowly added drop by drop, unceasingly stir, transfer and dilute to a 50 mL dry volumetric flask after it is cooled. It should be prepared immediately before use.

Note: acetyl chloride is a pungent reagent; maintain stirring when preparing methanol solution of acetyl chloride so as to prevent splashing; pay attention to protection.

4.5 Sodium carbonate solution

Weigh accurately 6 g exsiccated sodium carbonate (4.1) to a 100 mL beaker, dissolve it with water, transfer and dilute with water to a 100 mL volumetric flask.

4.6 Standard substance of triglyceride fatty acid

The purity ≥99 %; for category of fatty acids, refer to the Appendix A, table A.1.

4.7 Standard working solution of triglyceride fatty acid

Prepare standard working solution with appropriate concentration according to the contents of various fatty acids in the test sample, dilute to volume with toluene and store at a -10□ refrigerator; its shelf life is three months.

5 Apparatus and Equipments

5.1 Analytical balance: with a reciprocal sensibility of 0.01 g and 0.1 mg.

5.2 Water bath with constant temperature

5.3 Centrifuge separator: with a rotary speed over 5000r/min.

5.4 Gas chromatograph with an FID detector

5.5 Freeze-drying apparatus

5.6 Pressured gas blowing concentrators

5.7 Glass tubes with screw cap (with screw caps with polytetrafluoroethylene pad), 15mL

5.8 Centrifuge Tube

6 Analytical procedures

6.1 Treatment of the test sample

6.1.1 When the water content of the test sample is more than 5 %, freeze drying the test sample until the water content is less than 5 %.

6.1.2 Weigh 0.5 g test sample (accurate to 0.1 mg) to a 15 mL dry glass tube with a screw cap (5.7), and then add 5.0 mL toluene (4.2).

6.1.3 Weigh 0.2 g butter test sample (accurate to 0.1 mg) to a 15 mL dry glass tube with a screw cap (5.7), and then add 5.0 mL toluene (4.2).

6.2 Methanol esterification-extraction

6.2.1 Preparation of the test sample solution

Add 6.0 mL 10 % methanol solution of acetyl chloride (4.4) to the test sample (6.1.2 or 6.1.3), screw up the cap after it is filled with nitrogen gas, oscillation mixing and incubated in a 80□±1℃ water bath for 2h, shake it once every 20 min, and then take it out of the water bath and cool to room temperature. Transfer the reaction solution to a 50 mL centrifuge tube, wash the glass tube for three times with 3.0 mL sodium carbonate solution (4.5), combine the sodium carbonate solution (4.5) to a 50 mL centrifuge tube(5.8), mix even, and then centrifuge at 5000 r/min for 5 min. Take the supernatant of the upper layer as test solution which is then determined with gas chromatograph.

6.2.2 Preparation of standard solution

Accurately pipette 0.5 mL standard working solution of triglyceride fatty acid (4.7) to a 15 mL glass tube with screw cap (5.1), add 4.5 mL toluene, the other operation procedures are the same as those in (6.2.1).

6.3 Reference chromatographic conditions

Chromatographic column: with 100% dicyanopropyl polysiloxane as stationary liquid, 100 m×0.25 mm, 0.20 μm, or a chromatographic column with equivalent performance.

Carrier gas: nitrogen gas.

Flow rate of carrier gas: 1.0 mL /min.

Temperature of the injection port: 260℃

Split ratio: 30: 1

Temperature of the detector: 280℃.

Temperature of the column oven: the initial temperature is 140℃, maintain at this temperature for 5 min, increased the temperature to 240℃ at the rate of 4℃/min and then maintain at this temperature for 15 min.

6.4 Determination of the test sample solution

Accurately pipette and inject no less than two portions of 1.0 μL standard solution (6.2.2) and test solution 6.2.1), integrate and quantitate it according to chromatographic peak area.

7 Calculation and expression of results

7.1 Calculate the contents of various fatty acids in the test sample according to formula (1):

$$X_i = \frac{A_{si} \times C_{stdi} \times F_j}{A_{stdi} \times m} \times 100 \quad \cdots\cdots\cdots\cdots (1)$$

Where,

X_i – the contents of various fatty acids in the test sample, with a unit of mg/100g;

A_{si} - the peak areas of various fatty acids in the test sample solution;

C_{stdi} – the mass of standard substance in the standard working solution of triglyceride fatty acids (6.2.2), with a unit of mg;

F_j - the conversion factor of triglyceride fatty acids transforming to fatty acids, see the tableA.1 in Appendix A;

A_{stdi} - peak areas of various fatty acids in the standard solution;

m - the mass of the test sample, with a unit of g;

Expression of results: the determination results are expressed with arithmetic means parallel determined; truncate it to three significant figures.

7.2 Content of total fatty acids in the test sample

Calculate the content of total fatty acids in the test sample according to formula (2):

$$X_{Total\ FA} = \sum X_i \qquad \cdots\cdots\cdots\cdots (2)$$

Where,

$X_{Total\ FA}$ - the content of total fatty acids in the test sample, with a unit of mg/100g;

X_i - the contents of various fatty acids in the test sample, with a unit of mg/100g;

The determination results are expressed with arithmetic means parallel determined and truncated to it to three significant figures.

7.3 Calculate the percentage (%) Y of a certain fatty acid of total fatty acids in the test sample according to formula (3):

$$Y = X_i / X_{Total\ FA} \text{ 或 } Y = A_{si} \times F_j / \sum (A_{si} \times F_j) \quad \cdots\cdots\cdots\cdots (3)$$

8 Precision

The absolute error of the results of two independent determinations acquired under repeatability conditions shouldn't exceed 10% of the arithmetic mean.

9 Others

For the lowest detection limit of this method, see the tableA.1 in Appendix A.

The 2nd Method: Ammonia water - ethanol extraction method

10 Principles

After saponification treatment, the fat in milk and dairy products will generate free fatty acids, which will have methanol esterification reaction under catalysis of boron trifluoride; separate the fatty acids esterified with methanol with gas chromatographic column, detect them with a hydrogen flame ionization detector and then quantitate them with an external standard method.

11 Reagents and materials

Unless otherwise specified, all reagents used are analytical or above; and the water is 1st grade water specified in GB/T 6682.

11.1 Methanol, Chromatography pure

11.2 Aether

11.3 Petroleum ether, with the boiling range of 30□- 60□

11.4 Ethanol, ≥ 95% (v/v)

11.5 Ammonia water, 25 % (v/v)

11.6 N-hexane (C_6H_{14}), chromatographic pure

11.7 Taka-diastase, 128 U/mg

11.8 Methanol solution of boron trifluoride, 14% (w/v)

11.9 Saturated sodium chloride solution

Dissolve 360 g sodium chloride (NaCl) into 1.0 L water, stir to dissolve it; after it is clear, store it for later use.

11.10 Methanol solution of potassium hydroxide [c (KOH)＝0.5 mol/L]

Weigh 2.8 g potassium hydroxide (KOH), dissolve with methanol (11.1), dilute to 100 mL, and mix even.

11.11 Pyrogallolum methanol solution (10%, w/v)

Weigh 1.0 g Pyrogallolum and dissolve it with 10 ml methanol. The final concentration of the solution is 10% (w/v). Store it for later use.

11.12 Standard substance of fatty acid methyl ester

Purity ≥99%, Store it in a refrigerator with temperature lower than -18℃. Fatty acids refer to tableA.1 in Appendix A.

11.13 Standard working solution of fatty acid methyl ester

Prepare test samples with appropriate concentrations according to the contents of various fatty acids and the category of the fatty acids to be tested, dilute to volume with N-hexane and store it in a refrigerator with temperature lower than -10 □; its shelf life is three months.

12 Apparatus

12.1 Analytical balance: with a reciprocal sensibility of 0.1 mg.

12.2 Liposuction tube, 100 mL test tube with a ground stopper, drying to constant weight

12.3 Rotary evaporator

12.4 Centrifuge, with the rate above 5000 r/min

12.5 Thermostatic water bath

12.6 Gas chromatograph, with an FID detector

13 Analytical procedures

13.1 Preparation of test samples

Take the test samples out of the refrigerator and warm up to room temperature.

13.1.1 Liquid test samples

Weigh accurately 10 g (accurate to 0.1mg) sample to a liposuction tube for later detection

13.1.2 Solid state test samples

13.1.2.1 Samples containing starch

Weigh 1.0 g test sample (accurate to 0.1 mg) to a liposuction tube (12.2), add 0.1 g Taka-diastase (11.7) into it, dissolve with 10 mL distilled water of 45℃ - 50℃, after mixing completely, degas with nitrogen gas, put on the stopper, store in a 45□±1℃ drying oven for 30 min, and then take it out.

13.1.2.2 Sample containing no starch

Weigh 1.0 g test sample (accurate to 0.1 mg) to a liposuction tube (12.2), dissolve the test sample with 10mL distilled water of 65□±1□, shake even to completely disperse the sample.

Add 2 mL ammonia water (11.5) to the above-mentioned sample (13.1.1 and13.1.2), place into 65□±1□ water bath for 15 min, take out and shake slightly, and then cool to room temperature.

13.1.2.3 Butter

Weigh accurately 0.2 g test sample (accurate to 0.1 mg) to a flat bottom ground flask, esterified the content with esterication-saponification according to the method in (13.3).

13.2 Fat extraction

Add 10 mL ethanol (11.4) to prepared sample, and mix even. Add 25 mL of aether (11.2), put on the stopper and then shake for 1 min. Add 25 mL petroleum ether (11.3), put on the stopper and then shake for 1 min, allow to stand to separate into two layers. Transfer the organic layer to a flat bottom ground flask. Add 25 mL aether (11.2) and 25 mL petroleum ether (11.3), put on the stopper and shake for 1 min,

allow to stand to separate into two layers. Transfer the organic layer to a flat bottom ground flask, repeat the operation from “Add 25 mL anhydrous aether ….. allow to stand to separate into two layers.” Combine the extraction solution to a flat bottom ground flask, concentrate to dry with a rotary evaporator.

13.3 Esterication-saponification

Add 10 mL methanol solution of Pyrogallolum (11.11) to the concentrate (13.2) or butter (13.1.3). after concentration and drying, add 10 mL methanol solution of potassium hydroxide (11.10), and then keep a 80□±1□ water bath with reflux for 5 min - 10 min. Add 5 mL methanol solution of boron trifluoride (11.8), and continue to reflux for 15 min. Cool to room temperature, transfer the fluid in the flat bottom flask to a 50 mL centrifuge tube, wash the flat bottom flask with 3 mL saturated sodium chloride solution (11.9) for three times, combine the saturated sodium chloride solution to a 50 mL centrifuge tube, add 10 mL N-hexane (11.6), shake slightly, centrifuge at 5000 r/min for 5 min, and then take the supernatant of the upper layer as test solution and determined with gas chromatograph (12.6).

Note Methanol solution of boron trifluoride is a strong corrosive reagent, pay attention to protection when using it.

13.4 Chromatography reference conditions

Chromatographic column: with 100% dicyanopropyl polysiloxane as stationary liquid, 100 m×0.25 mm, 0.20 μm, or a chromatographic column with equivalent performance.

Carrier gas: nitrogen gas.

Flow rate of carrier gas: 1.0 mL /min.

Temperature of the injection port: 260 □.

Split ratio: 30: 1.

Temperature of the detector: 280 □.

Temperature of the column oven: the initial temperature is 140 □, maintains at this temperature for 5 min, increased the temperature to 240 □ at the rate of 4□/min and then maintain at this temperature for 15 min.

Injection volume: 1.0 μL

13.5 Determination

Accurately pipe and inject no less than two portions of 2 μL standard working solution (3.2.12) and test solution (3.4.3) of fatty acid methyl ester, integrate and quantitate it according to chromatographic peak area. For typical chromatograms, refer to appendix A.

14 Calculation and expression of results

14.1 Calculation of the contents of various fatty acids in the test sample

Calculate the contents of various fatty acids in the test sample according to formula (4):

$$X_i = \frac{A_{si} \times C_{stdi} \times V \times F_i}{A_{stdi} \times m} \times 100 \quad \cdots\cdots (4)$$

Where,

X_i – the contents of various fatty acids in the test sample, with a unit of mg/100g;

A_{si} - the peak areas of various fatty acid methyl esters in the test sample solution;

C_{stdi} – the concentrations of various fatty acid methyl esters in the standard working solution of fatty acid methyl ester, with a unit of mg/mL;

V – the volume of diluted solution, with a unit of mL;

A_{stdi} - the peak areas of various fatty acid methyl esters in the mixed standard working solution;

F_i – the conversion factor of fatty acid methyl esters transforming to fatty acids, see table A.1 in the Appendix A;

m – The mass of the test sample, with a unit of g.

The determination results are expressed with arithmetic means parallel determined; truncate the result to three significant figures.

14.2 Calculation of the contents of total fatty acid in the test sample

Calculate the content of total fatty acid in the test sample according to formula (5):

$$X_{TotalFA} = \sum X_i \quad \cdots\cdots (5)$$

Where,

$X_{Total\ FA}$ - the content of total fatty acid in the test sample, with a unit of mg/100g;

X_i - the contents of various fatty acids in the test sample, with a unit of mg/100g;

The determination results are expressed with arithmetic means parallel determined and truncated to three significant figures.

14.3 Calculation the percentage (%) of a certain fatty acid of the total fatty acids in the test sample

The percentage (%) Y of a certain fatty acid of the total fatty acids in the test sample is calculated according to formula (6):

$$Y = \frac{X_i}{X_{TotalFA}} \times 100 \quad 或 Y = \frac{A_{zi} \times F_i}{\sum A_{zi} \times F_i} \times 100 \cdots\cdots (6)$$

15 Precision

The absolute error of two independent determination results acquired under repeatability conditions shouldn’t exceed 15 % of the arithmetic mean.

16 Others

For the lowest detection limit of this method, see tableA.1 in Appendix A.

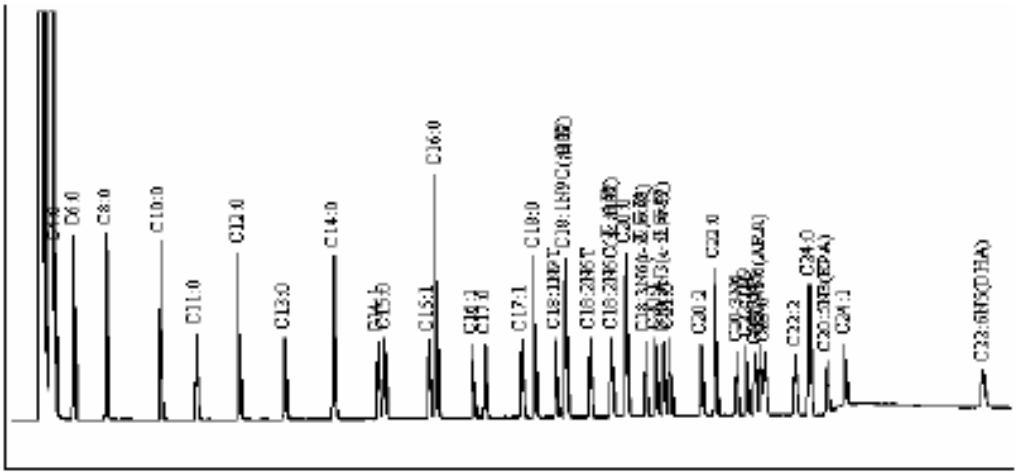
Appendix A

(Informative)

Typical spectrums of various fatty acids determined with gas chromatography and the conversion factors

A.1 the typical spectrums of standard solutions of 37 fatty acids

The typical spectrums of standard solutions of 37 fatty acids refer to the figure A.1.



Note: Different Columns may lead to different retention time of fatty acids. Please reference the retention time of the lab spectrums which calibrated from single standard

Figure A.1 the typical spectrums of standard solutions of 37 fatty acids

A.2 Summary of fatty acids, detection limit and the conversion factor of fatty acid methyl ester or triglyceride fatty acid transforming to fatty acid

The summary of fatty acids, detection limit and the conversion factor of fatty acid methyl ester or triglyceride fatty acid transforming to fatty acid please refer the tableA.1.

Table A.1 Summary of fatty acids, detection limit and the conversion factor of fatty acid methyl ester or triglyceride fatty acid transforming to fatty acid

No	Name of fatty acid	The lowest detection limit (mg/kg)	*Fi* conversion coefficient	*Fj* conversion coefficient
1	Butanoic acid (C4: 0)	5.0	0.8627	0.8742
2	Hexanoic acid (C6: 0)	5.0	0.8923	0.9016
3	Octanoic acid (C8: 0)	5.0	0.9114	0.9192
4	Decanoic acid (C10: 0)	5.0	0.9247	0.9314
5	Undecanoic acid (C11: 0)	5.0	0.9300	0.9363

6	Lauric acid (C12: 0)	5.0	0.9346	0.9405
7	Tridecylic acid (C13: 0)	5.0	0.9386	0.9442
8	Myristic acid tetradecanoic acid (C14: 0)	5.0	0.9421	0.9473
9	Myristoleic acid (C14: 1n5)	5.0	0.9417	0.9470
10	Pentadecanoic acid (C15: 0)	5.0	0.9453	0.9502
11	Pentadecenoic acid (C15: 1n5)	5.0	0.9449	0.9499
12	Palmitic acid (C16: 0)	5.0	0.9481	0.9529
13	Palmitoleic acid (C16: 1n7)	5.0	0.9477	0.9525
14	Heptadecanoic acid (C17: 0)	5.0	0.9507	0.9552
15	Heptadecenoic acid (C17: 1n7)	5.0	0.9503	0.9549
16	Stearic acid (C18: 0)	5.0	0.9530	0.9573
17	Elaidic acid (C18: 1n9t)	5.0	0.9527	0.9570
18	Oleic acid (C18: 1n9c)	5.0	0.9527	0.9571
19	Trans-linoleic acid (C18: 2n6t)	5.0	0.9524	0.9568
20	Linoleic acid (C18: 2n6c)	5.0	0.9524	0.9568
21	Arachidic acid (C20: 0)	5.0	0.9570	0.9609
22	γ - linolenic acid (C18: 3n6)	5.0	0.9520	0.9559
23	Eicosenoic acid (C20: 1)	5.0	0.9568	0.9608
24	α - linolenic acid (C18: 3n3)	5.0	0.9520	0.9560
25	Heneicosanoic acid (C21: 0)	5.0	0.9588	0.9628
26	Eicosadienoic acid (C20: 2)	5.0	0.9565	0.9605
27	Behenic acid (C22: 0)	5.0	0.9604	0.9642
28	Eicosatrienoic acid (C20: 3n6)	5.0	0.9562	0.9598
29	Erucic acid (C22: 1n9)	5.0	0.9602	0.9639
30	Eicosatrienoic acid (C20: 3n3)	5.0	0.9562	0.9598
31	Arachidonic acid ARA (C20: 4n6)	5.0	0.9560	0.9597
32	Tricosanoic acid (C23: 0)	5.0	0.9620	0.9658
33	Docosadienoic acid (C22: 2n6)	5.0	0.9600	0.9638
34	Tetracosanoic acid (C24: 0)	5.0	0.9963	1.0002
35	Eicosapentaenoic acid EPA (C20: 5n3)	10.0	0.9557	0.9592
36	Tetracosenoic acid (C24: 1n9)	10.0	0.9632	0.9666
37	Docosahexaenoic acid methyl ester DHA (C22: 6n3)	10.0	0.9590	0.9624

Note: F_i is the coefficient of fatty acid methyl ester transforming to fatty acid.

F_j is the coefficient of triglyceride fatty acid transforming to fatty acid.