National Standard of the People's Republic of China

GB 5413.36—2010

National Food Safety Standard
Determination of trans fatty acids in foods for infants and young children, milk and milk products

Issued on 2010-03-26 Implemented on 2010-06-01

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Preface

Appendix A of this standard is informative appendix.

This standard is issued for the first time

National Food Safety Standard
Determination of trans fatty acids in foods for infants and young children, Milk and milk products

1. Scope

This standard specifies the method for the determination of trans fatty acid in foods for infants and young children, milk and milk products.

This standard applies to the determination of trans fatty acid in foods for infants and young children, milk and milk products.

2. Normative references

The reference document of this standard is essential to the application. For dated reference documents, only the date of the release note applies to this standard. For undated reference documents, the latest versions (including all amendments) apply to this standard.

3. Principle

The fat in the sample is extracted by organic solvents. Under the alkaline condition, the extracts react with the methanol and the fatty acid methyl esters are obtained. Gas chromatograph with hydrogen flame ionization detector is used to separate cis and trans fatty acid methyl esters and quantitatively analyze the content of trans fatty acid by external reference method.

4. Reagents and materials

If not specified, all reagents used in this method are analytical reagents, and water is the 1st grade water specified by GB/T6682.

4.1 Petroleum ether: Boiling range: 30□-60□.

4.2 Ethyl ether（$C_4H_{10}O$）.

4.3 Ethanol（C_2H_6O）: 95% volume fraction.

4.4 Hexane（C_6H_{14}）: chromatographic purity

4.5 Ammonia（$NH_3 \cdot H_2O$）: 25-28 %.

4.6 Potassium hydroxide（KOH）.

4.7 Methanol（CH_4O）.

4.8 Amylase: Activity unit: 1.5 U/mg, the dosage depends on the activity unit.

4.9 Anhydrous sodium sulfate（Na_2SO_4).

4.10 Potassium hydroxide – methanol solution (4 mol/L)

Dissolve 26.4g potassium hydroxide with 80mL methanol, then cool it to room temperature, and predetermine to 100mL with methanol. Add about 5g anhydrous sodium sulfate (4.9) into above solution, stir evenly and filter, keep the filtrate.

4.11 Fatty acid methyl ester standard

Methyl octadecanoate (C18:0), trans-9-methyl octadecanoate (C18:1-9t), cis-9-methyl octadecanoate (C18:1-9c), trans-9, 12-methyl octadecadienoate (C18:2 9t, 12t), cis-9, 12- methyl octadecadienoate (C18:2-9c, 12c). Store them in the Refrigerator below -15□.

4.12 Standard stock solutions of trans fatty acid methyl esters (10.0 mg/mL)

Dissolve 500 mg (accurate to 0.1 mg) trans-9-methyl octadecanoate and trans-9, 12-methyl octadecadienoate standard and dilute to 50 mL with normal hexane respectively. Store at -15□.

4.13 Standard Medium Solution of trans fatty acid methyl esters (1.0 mg/mL)

Pipette 10.0 mL of the two standard stock solutions of trans fatty acid methyl esters (4.12) into 100mL volumetric flask respectively, and dilute to 100 mL with hexane (4.4). The solution should be prepared before use. It's used to test the peak point of standard curve.

4.14 Standard working solutions

Prepare them before use. Pipette 0, 2.0, 4.0, 6.0, 8.0, and 10.0 mL of the standard solution (4.13) into the 10mL volumetric flasks, and dilute with hexane respectively. The concentrations of the solutions are 0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL respectively.

4.15 Mixture solution of the standard fatty acids methyl esters

Dissolve and dilute the fatty acids methyl esters standard (4.11) with hexane. Each component concentration should be 0.05 mg/mL to 0.5 mg/mL. It is used to identify the separation degree and qualification of cis and trans fatty acid methyl esters.

4.16 Congo red solution

Dissolve 1g of Congo red and dilute to 100 mL with distilled water.

5. Equipments

5.1 Gas chromatography: with hydrogen flame ionization detector.

5.2 Rotary evaporator.

5.3 Constant temperature water bath:40 □～80 □。

5.4 Vortex oscillator

5.5 Centrifuge, speed ≥4000rpm

5.6 Mojonnier extraction flask

5.7 Mojonnier ether extraction bottle shaker.

5.8 Ether collection flask: round bottle flask, in equipped with rotary evaporator

5.9 Balance: with 0.1 mg sensitivity.

6. Analytical procedure

6.1 Sample treatment

6.1.1 Starch-containing sample

Weigh about 1.5g solid sample that has been mixed evenly or about 5g of liquid sample (accurate to 0.1 mg) into the Mojonnier ether extraction bottle, add about 0.1g of amylase (enzyme activity 1.5 U/mg), and mix evenly. Add 8 mL ~10 mL water of 45□±2□, shake evenly and plug on; incubate in the water bath at 55 □±2 □ (5.3) for 2 h. Shake every 10 minutes. To test whether the starch is hydrolyzed completely, add two drops 0.1mol/L iodine solution. If there is no blue color, it indicates that the sample hydrolyzed completely; otherwise keep the ether extraction flask incubate in the water bath until the blue color disappears. Take out the Mojonnier ether extraction flask and cool it to room temperature.

6.1.2 Starch-free sample

Weigh about 1.5g of solid sample that has been mixed fully or about 10g of liquid sample (accurate to 0.1 mg) into the Mojonnier ether extraction flask (5.6), add 10mL water of 45□±2 □, and elute the sample into the small ball of the Mojonnier fat extraction flask (5.6). Mix evenly until the sample is dissolved. Cool it to room temperature.

6.1.3 Extraction of fat

Add 3.0mL of ammonia (4.5) into the Mojonnier ether extraction flask (5.6), and mix well. Incubate it into the water bath (5.3) of 60□±2 □ for 15 min~20 min. Cool it to room temperature. Add 10mL of the ethanol (4.3) and 1 drop of the Congo red solution (4.16), and mix well. Add 25.0mL of ethyl ether (4.2), seal with cork, and vortex it on the Mojonnier ether extraction flask shaker (5.6) for 1min or shake it handily. Add 25mL the petroleum ether (4.1), shake it for 1 min and then centrifuge at the speed≥4000 rpm. Remove the supernatant into the fat collection bottle (5.8). This is the first extraction. Add 5mL ethanol, 25mL ethyl ether, and 25mL petroleum ether into the residual sample solution, and extract it as the same method mentioned above. Remove the supernatant after the centrifugation (5.5), and merge it with the first extraction. Put the ether collection flask (5.8) in rotary evaporator (5.2) for rotary evaporation with nitrogen gas at 60□±2 □ to remove the solvent and maintain the fat residue.

6.1.4 Preparation for fatty acid methyl ester

Dissolve the fat mentioned above with 10 mL of hexane (4.4), and pipette 3.0 mL into a 10 mL tube with

plug; pipette 0.3mL potassium hydroxide-methanol solution (4.10). Plug on, and shake fully on the vortex shaker (5.4) for 2 min. Centrifuge at 4000 rpm for 5 min, and transfer the supernatant into GC test sample bottle. This is the test sample solution.

6.2 Determination

6.2.1 Reference chromatography condition

Chromatographic column: Capillary column which the filling material is cyanpropyl aryl polysiloxane, the length is 100 m, the inside diameter is 0.25 mm, and the membrane thickness is 0.2 μm; or other chromatographic column with equivalent function performance.
Injector temperature: 250□; Carrier gas: (N_2)
Detector temperature: 300□;
Splitting ratio: 10: 1;
Injection volume: 1.0 μL.
Temperature programming: see Table 1。

Table 1 Temperature programming

Heating rate (□/min)	Target temperature (□)	Retention time (min)
Initial temperature	120	0
10	175	10
5	210	5
5	230	5

6.2.2 Standard curve.

Under the optimal working condition of equipment, inject the series of standard working solutions (4.14) respectively. Plot the standard curve, with peak area as y-axis, and the concentration of standard working solution as x-axis.

6.2.3 Identification of trans fatty acid methyl ester:

Inject the mixed standard solution of fatty acid methyl ester (4.15) for identification of separation degree of cis- and trans- fatty acid methyl ester and qualification. The positions of peaks of trans methyl octadecanoate and trans methyl octadecadienoate should comply with figure A.1 in the appendix A.

6.2.4 Determination of sample solution

Inject the sample solution into the gas chromatograph. The position of peak of trans fatty acid methyl ester in sample solution is referred figure A.1 in the appendix A. Determine the total peak area in the areas of C18:1t and C18:2t; consult the standard curve to obtain the mass fraction of trans methyl

octadecanoate and trans methyl octadecadienoate in the sample solution.

7. Result calculation and presentation

The content of trans octadecanoic acid and trans octadecadienoic acid in sample are represented by X_1 and X_2 respectively, they should be calculated as formula (1) respectively:

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

Where:

$X_{(1or2)}$—— the content of trans octadecanoic acid or trans octadecadienoic acid in sample, mg/100g

V —— the constant volume of the sample solution, mL

m—— mass of sample, g

c_i—— the mass concentration of trans methyl octadecanoate or trans methyl octadecadienoate in the sample solution, mg/mL;

M_{ai}—— the molecular weight of trans octadecanoic acid or trans octadecadienoic acid;

M_{bi} ——the molecular weight of trans methyl octadecanoate or trans methyl octadecadienoate.

The total content of trans fatty acids in sample X, should be calculated as formula (2):

$$X = X_1 + X_2 \quad \cdots\cdots (2)$$

Where:

X——the total content of trans fatty acids, mg/100g;

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

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

The calculated result is represented by the arithmetic mean from two independent results of determination, and should display in 3 significant figures.

8. Precision

The absolute difference between the two independent determination results that are obtained under repeated conditions should not exceed 10% of the arithmetic mean.

9. Others

The detection limit of this standard is 30 mg/kg.

Appendix A

(Informative appendix)

Chromatogram of mixed standard solution of trans fatty acids

A.1 Chromatogram of mixed standard solution of trans fatty acids

The Chromatogram of mixed standard solution of trans fatty acids, please refer to the figure A.1

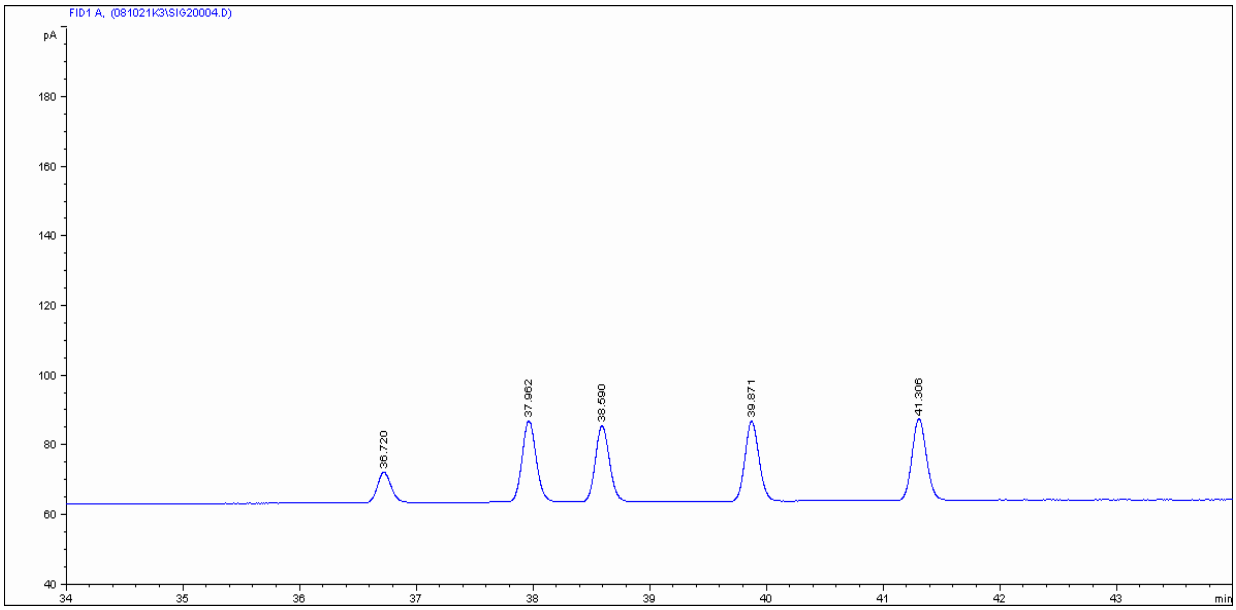

Figure A.1: Chromatogram of mixed standard solution of trans fatty acids