



**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB 5009.181-2016

**National food safety standard - Determination of
malondialdehyde in food**

食品安全国家标准 食品中丙二醛的测定

(English Translation)

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Table of Contents

Foreword...	3
1 Scope...	4
2 Principles...	4
3 Reagents and materials...	4
4 Instruments and equipment...	5
5 Analytical procedures...	6
6 Analysis results expression...	7
7 Precision...	7
8 Principles...	7
9 Reagents and materials...	8
10 Instruments and equipment...	9
11 Analytical procedures...	9
12 Analysis results expression...	9
13 Precision...	10
14 Other...	10

1 Scope

This Standard specifies the method for determination of malondialdehyde in food.

Method 1 of this Standard is applicable to the determination of malondialdehyde in food.

Method 2 of this Standard is applicable to the determination of malondialdehyde in animal and vegetable fats and oils.

Method 1 High performance liquid chromatography

2 Principles

The sample is first extracted with acid solution. Then the extract is reacted with thiobarbituric acid (TBA) to form a colored compound. USE high performance liquid chromatography-diode array detector to determine; and external standard method to quantify.

3 Reagents and materials

Unless otherwise stated, the reagents used in this method are of analytically pure; the water is Grade I water specified in GB/T 6682.

3.1 Reagents

3.1.1

Methanol (CH_3OH). chromatographically pure.

3.1.2

Trichloroacetic acid ($\text{C}_2\text{HCl}_3\text{O}_2$).

3.1.3

Ethylene diamine tetraacetic acid ($\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8 \cdot 2\text{H}_2\text{O}$).

3.1.4

Thiobarbituric acid (TBA) ($\text{C}_4\text{H}_4\text{N}_2\text{O}_2\text{S}$).

3.2.1 Ammonium acetate solution (0.01 mol/L). WEIGH 0.77 g of ammonium

acetate; ADD water to dissolve and dilute to 1000 mL; and USE a 0.45 μm filter to filter.

3.2.2 Trichloroacetic acid mixture. accurately WEIGH 37.50 g (accurate to 0.01

g) of trichloroacetic acid (3.1.2) and 0.50 g (accurate to 0.01 g) of ethylene diamine tetraacetic acid (3.1.3); USE water to dissolve, dilute to 500 mL.

3.2.3 Aqueous solution of thiobarbituric acid (TBA). accurately WEIGH 0.288 g

(accurate to 0.001 g) of thiobarbituric acid (3.1.4) dissolved in water and diluted to 100 mL (If not easy to dissolve, it can be heated by ultrasonic until completely dissolved. After cooling, DILUTE to volume of 100 mL), equivalent to 0.02 mol/L.

3.3 Standard

1,1,3,3-tetraethoxypropane (also known as malondialdehyde acetal, $\text{C}_{11}\text{H}_{24}\text{O}_4$, CAS number. 122-31-6). Purity $\geq 97\%$.

3.4.1 Standard stock solution of malondialdehyde (100 $\mu\text{g/mL}$). Accurately

TRANSFER 0.315 g (accurate to 0.001 g) of 1,1,3,3-tetraethoxypropane to a 1000 mL volumetric flask; after dissolving in water, DILUTE to 1000 mL; SET in a refrigerator at 4 $^{\circ}\text{C}$ for storage. It is valid for 3 months.

3.4.2 Standard use solution of malondialdehyde (1.00 µg/mL). accurately

PIPETTE 1.0 mL of standard stock solution of malondialdehyde (3.4.1); USE trichloroacetic acid mixture (3.2.2) to dilute to 100 mL; SET in a refrigerator at

4 °C for storage. It is valid for 2 weeks.

3.4.3 Standard series solution of malondialdehyde. accurately PIPETTE 0.10

mL, 0.50 mL, 1.0 mL, 1.5 mL, 2.5 mL of standard use solution of malondialdehyde in 10 mL volumetric flasks; ADD trichloroacetic acid mixture (3.2.2) to dilute to volume. The concentration of the series of standard solutions is 0.01 µg/mL, 0.05 µg/mL, 0.10 µg/mL, 0.15 µg/mL, 0.25 µg/mL. Prepared when used.

4.1 High performance liquid chromatography. equipped with a diode array

detector.

4.2 Balance. The sensitivity is 0.0001 g, 0.01 g.

4.3 Thermostatic oscillator.

4.4 Thermostatic water bath.

to the standard curve, the concentration of malondialdehyde in the solution to be tested is obtained. The liquid chromatogram of malondialdehyde standard is shown in Appendix A.

6 Analysis results expression

The content of malondialdehyde in the sample shall be calculated according to formula (1).

Where.

X - The content of malondialdehyde in the sample, in milligrams per kilogram (mg/kg);

c - The concentration of malondialdehyde in the sample solution obtained from the standard series curve, in micrograms per milliliter (µg/mL);

V - The constant volume of sample solution, in milliliters (mL);

m - The mass of sample represented by the final sample solution, in grams (g);

1000 - Conversion factor.

The calculation result is expressed as the arithmetic mean of the two independent determination results obtained under repeated conditions. The result retains two significant figures.

7 Precision

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

Method 2 Spectrophotometry

8 Principles

After the malondialdehyde is extracted by trichloroacetic acid solution, it reacts with thiobarbituric acid (TBA) to form a pink compound. Its absorbance at 532 nm of wavelength is measured and compared with the standard series to to dilute to volume. The concentration of the series of standard solutions is 0.01

μg/mL, 0.05 μg/mL, 0.10 μg/mL, 0.15 μg/mL, 0.25 μg/mL. Prepared when used.

10 Instruments and equipment

10.1

Spectrophotometer.

10.2

Balance. The sensitivity is 0.0001 g, 0.01 g.

10.3

Thermostatic oscillator.

10.4

Thermostatic water bath.

11.1 Sample preparation

WEIGH 5 g (accurate to 0.01 g) of the sample; PLACE it in a 100 mL conical flask with stopper; accurately ADD 50 mL of trichloroacetic acid mixture; SHAKE it well; and USE the stopper to seal. PLACE on a thermostatic oscillator to shake at 50 °C for 30 min; REMOVE, and COOL to room temperature. USE double-layer quantitative slow filter paper to filter; DISCARD the primary filtrate;

and the subsequent filtrate is standby.

Accurately PIPETTE 5 mL of the above filtrate and of standard series solution respectively; PLACE them in 25 mL colorimetric tube with stopper. TAKE another 5 mL of trichloroacetic acid mixture as a sample blank; ADD 5 mL of aqueous solution of thiobarbituric acid (TBA) respectively; COVER the stopper;

and MIX well. PLACE in a 90 °C water bath to react for 30 min; TAKE out; and