



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

GB 5009.179-2016

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

食品安全国家标准 食品中三甲胺的测定

(English Translation)

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1 Scope

This standard specifies methods for the determination of trimethylamine in aquatic animals and their products as well as meat and meat products.

This standard applies to the determination of trimethylamine in aquatic animals and their products as well as meat and meat products.

Method I - Headspace gas chromatography-mass spectrometry

2 Principle

The specimen is extracted by the 5% trichloroacetic acid solution, the extract is placed in a sealed headspace bottle. The trimethylamine hydrochloride is converted to trimethylamine under the action of an alkali solution, it is equilibrated at 40 °C for 40 min. Trimethylamine reaches the dynamic equilibrium in both the gas and liquid phases. The gas in the headspace bottle is taken and injected into the gas chromatograph-mass spectrometer for testing.

The retention time (RT), auxiliary qualitative ions (m/z 59 and m/z 42) and quantitative ions (m/z 58) are used for qualitative; the external standard method is used for quantitation.

3 Reagents and materials

Unless otherwise stated, the reagents used in this method are of analytical grade and the water is the grade I water as specified in GB/T 6682.

3.1 Reagents

3.1.1

Sodium hydroxide (NaOH).

3.1.2

Trichloroacetic acid ($C_2HCl_3O_2$).

3.2 Preparation of reagent

3.2.1 50% sodium hydroxide solution. WEIGH 100 g of sodium hydroxide;

DISSOLVE it in 100 mL of water at 20 °C ~ 30 °C.

3.2.2 5% trichloroacetic acid solution. WEIGH 25 g of trichloroacetic acid;

DISSOLVE it in water; MAKE its volume reach to 500 mL.

3.3 Standard substance

Trimethylamine hydrochloride (CAS. 593-81-7), molecular formula. $(CH_3)_3NHCl$, purity $\geq 98\%$, which is placed in a desiccator and preserved at 4 °C.

3.4.1 Trimethylamine standard stock solution. WEIGH 0.0162 g of

trimethylamine hydrochloride standard substance; USE the 5% trichloroacetic acid solution to dissolve and dilute it to 100 mL, which is equivalent to the trimethylamine standard stock solution of concentration 100 $\mu g/mL$;

PRESERVE it at 4 °C conditions.

3.4.2 Trimethylamine standard use solution. TAKE a certain volume of

trimethylamine standard stock solution; USE the 5% trichloroacetic acid solution to dilute it stepwise to the trimethylamine standard use solution which has a concentration of 1.0 $\mu g/mL$, 2.0 $\mu g/mL$, 5.0 $\mu g/mL$, 10.0 $\mu g/mL$, 20.0

$\mu g/mL$, 40.0 $\mu g/mL$, respectively.

4 Instruments and equipment

4.1

Gas chromatography-mass spectrometer. It is equipped with split/splitless sample injection inlet and electron impact ionization source (EI source).

4.2

Balance. The sensitivities are 0.1 mg and 1 mg, respectively.

4.3

Constant-temperature water bath. Temperature control accuracy is ± 2 °C.

4.4

Headspace bottle. The volume is 20 mL, equipped with Teflon silicone rubber pad and sealing cap, baked at 120 °C for 2 h before use.

4.5

Micro-syringe. 1 mL.

4.6

Medical plastic syringe. 5 mL.

4.7

Homogenizer.

4.8

Meat grinder.

4.9

Low-speed centrifuge.

b) Carrier gas. high purity helium;

c) Flow rate 1.0 mL/min; inlet temperature 220 °C;

d) Split ratio. 10.1;

e) Temperature-rise program. hold at 40 °C for 3 min, increase to 220 °C at the rate of 30 °C/min, maintain for 1 min.

5.2.2 Mass spectrometry conditions

The mass spectrometry conditions are as follows.

a) Ion source. electron impact ionization source (EI source), temperature.

220 °C;

- b) Ionization energy. 70 eV;
- c) Transmission line's temperature. 230 °C;
- d) Solvent delay. 1.5 min;
- e) Scanning mode. Selective ion monitoring (SIM).

5.3.1 Headspace sample injection. EQUILIBRATE the prepared specimen at

40 °C for 40 min. Under the chromatographic mass spectrometry condition of

5.2, USE a syringe to extract 100 µL of the gas above the solution in the headspace bottle; INJECT it into a GC-MS for determination.

5.3.2 Qualitative determination. USE the selective ion monitoring method to

collect data; USE the retention time (RT) of trimethylamine in sample solution, auxiliary qualitative ions (m/z 59 and m/z 42), quantitative ions (m/z 58), the kurtosis ratio (Q) of the auxiliary qualitative ions to quantitative ions, to perform the comparative qualitiveness with the standard solution. The relative deviation between the kurtosis ratio (Q sample) of the auxiliary qualitative ion to the quantitative ion of trimethylamine in the sample solution and the kurtosis ratio (Q standard) of the auxiliary qualitative ion to the quantitative ion of trimethylamine in the standard solution is controlled within $\pm 15\%$.

5.3.3 Quantitative determination. USE the external standard method for

quantification. USE the peak area of trimethylamine in the standard solution as the ordinate and the concentration of trimethylamine in the standard solution as the abscissa, DRAW the standard curve; USE the calibration curve to calculate the concentration of trimethylamine in the sample solution.

10.4.1 Trimethylamine standard stock solution. WEIGH 0.0162 g of

trimethylamine hydrochloride standard substance; USE the 5% trichloroacetic acid solution to dissolve and dilute it to 100 mL, which is equivalent to the trimethylamine standard stock solution of concentration 100 µg/mL;

PRESERVE it at 4 °C conditions.

10.4.2 Trimethylamine standard use solution. TAKE a certain volume of

trimethylamine standard stock solution; USE the 5% trichloroacetic acid solution to dilute it stepwise to the trimethylamine standard use solution which has a concentration of 1.0 µg/mL, 2.0 µg/mL, 5.0 µg/mL, 10.0 µg/mL, 20.0 µg/mL, 40.0 µg/mL, respectively.