



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

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National food safety standard - Food additives - DL-Alanine

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1 Scope

This Standard is applicable to the food additive DL-alanine, which is obtained through enzymatic racemization, extraction and refining with L-alanine as the raw material.

2 Chemical Name, Molecular Formula, Structural

Formula and Relative Molecular Mass

2.1 Chemical Name

D-2-alanine, L-2-alanine

2.2 Molecular Formula

C₃H₇NO₂

2.4 Relative Molecular Mass

89.09 (in accordance with the international relative atomic mass of 2018)

3.1 Sensory Requirements

The sensory requirements shall comply with the requirements of Table 1.

Table 1 -- Sensory Requirements

D-alanine L-alanine

Appendix A Inspection Method

A.1 General Rules When other requirements are not specified, the reagents and water used in this Standard refer to analytically pure reagents and Grade-3 water specified in GB/T 6682.

When other requirements are not specified, the standard solutions used in tests, and standard solutions, preparations and products used for impurity determination shall be prepared in accordance with the stipulations of GB/T 601, GB/T 602 and GB/T 603.

When it is not specified which solvent is used for preparation, the solutions used in tests refer to aqueous solutions.

A.2 Identification Test A.2.1 Reagent and material Ninhydrin solution: weigh-take 0.1 g of ninhydrin; use water to dissolve it and dilute to a constant volume of 100 mL.

A.2.2 Ninhydrin test Weigh-take about 0.1 g of specimen, accurate to 0.01 g; dissolve it in 100 mL of water.

Take 5 mL of this solution; add 2 mL of ninhydrin solution. Heat it up to boiling; after a certain time, it shall turn purple.

A.2.3 Infrared spectroscopy test Adopt the potassium bromide smear method, in accordance with GB/T 6040, determine the infrared absorption spectrum. The obtained infrared spectrogram shall be consistent with the spectrogram of DL-alanine standard substance (see Appendix B).

A.3 Determination of DL-alanine Content (calculated by dry basis)

A.3.1 Method summary The specimen takes anhydrous formic acid as the auxiliary solvent, glacial acetic acid as the solvent and crystal violet as the indicator. Use perchloric acid standard titration solution to titrate it. In accordance with the volume of the consumed standard titration solution of perchloric acid, calculate the content of DL-alanine.

A.3.2 Reagents and materials A.3.2.1 Anhydrous formic acid.

When titrating the specimen, if the difference between the temperature of the standard titration solution of perchloric acid and the temperature at the time of calibration exceeds 10 °C, then, it shall be re-calibrated; if it does not exceed 10 °C, then, in accordance with Formula (A.2), correct the concentration c of the standard titration solution of perchloric acid, expressed in (mol/L).

Where, c_0 ---the concentration of the standard titration solution of perchloric acid at the time of calibration, expressed in (mol/L);

α ---the expansion coefficient of glacial acetic acid;

T ---the actual temperature of the standard titration solution of perchloric acid when titrating

the specimen, expressed in (°C);

T0--the temperature of the standard titration solution of perchloric acid at the time of calibration, expressed in (°C).

The arithmetic mean value of parallel determination results shall prevail in the test result. The absolute difference between two independent determination results obtained under repeatability conditions shall not be greater than 0.3% of the arithmetic mean value.

A.4 Determination of Loss on Drying A.4.1 Instruments and equipment A.4.1.1 Electrically heated drying oven.

A.4.1.2 Weighing bottle.

A.4.1.3 Desiccator.

A.4.2 Analytical procedures Use a weighing bottle that has been dried to a constant mass to weigh-take 1 g ~ 2 g of specimen, accurate to 0.0001 g. Place it in a 105 °C $\pm$ 2 °C electrically heated drying oven to dry for 3 h, then, take it out, cover it and put it into a desiccator. Cool it down to room temperature (about 30 min), then, weigh it.

A.4.3 Result calculation The mass fraction w_2 of loss on drying in the specimen shall be calculated in accordance with Formula (A.3).

The determination result shall be accurate to one decimal place. The arithmetic mean value of parallel determination results shall prevail in the test result. The absolute difference between two independent determination results obtained under repeatability conditions shall not be greater than 0.05.

A.6 Determination of Transmittance A.6.1 Instrument and equipment Spectrophotometer.

A.6.2 Analytical procedures Weigh-take 10 g of specimen, accurate to 0.01 g. Place it in a 100 mL beaker; add 70 mL of water to dissolve it. Transfer it into a 100 mL volumetric flask and dilute to the scale; shake it well. Use a 1 cm cuvette, use water as the blank control; at the wavelength of 430 nm, determine the light transmittance of the specimen solution; record the reading.

The arithmetic mean value of parallel determination results shall prevail in the test result. The absolute difference between two independent determination results obtained under repeatability conditions shall not be greater than 0.2% of the arithmetic mean value.

A.7 Determination of Specific Rotation A.7.1 Reagent and material Hydrochloric acid solution: 1 + 1.

A.7.2 Instrument and equipment Polarimeter: equipped with sodium light (sodium spectrum D line 589.3 nm), with an accuracy of $\pm$ 0.01°.

A.7.3 Analytical procedures Weigh-take 10 g of specimen that has been pre-dried at 105 °C

to a constant mass, accurate to 0.0001 g. Place it in a 100 mL volumetric flask; add hydrochloric acid solution to dissolve it and dilute to a constant volume; shake it well. Adjust the temperature of the solution to 20 °C. Use the above-mentioned specimen solution to rinse the optical rotation tube for 3 times. Add the specimen solution (without bubbles) into the optical rotation tube; observe and determine the optical rotation.

A.7.4 Result calculation Specific rotation α_m (20 °C, D), expressed in (°) · dm² · kg⁻¹, shall be calculated in accordance with Formula (A.4).

A.8.4 Result determination The turbidity of the specimen solution is shallower than that of the control solution, i.e., the chloride content is less than or equal to 0.02%.

A.9 Determination of Burning Residue A.9.1 Reagents and materials A.9.1.1 Concentrated sulfuric acid.

A.9.1.2 Sulfuric acid solution: 1 + 8.

A.9.2 Instruments and equipment A.9.2.1 Platinum (or porcelain) crucible.

A.9.2.2 High-temperature furnace.

A.9.2.3 Desiccator.

A.9.3 Analytical procedures Weigh-take about 2 g of specimen, accurate to 0.0001 g. Place it in a crucible that has been burnt at 800 °C +/- 25 °C to a constant mass. Slowly dropwise add 1 mL of sulfuric acid solution to completely moisten the specimen. Firstly, heat it up on an electric furnace with a small fire, until the specimen has just begun to be carbonized, then, remove it and cool it down. Then, dropwise add 0.5 mL of concentrated sulfuric acid; use the above-mentioned method to heat it up, until the sulfuric acid vapor is exhausted; transfer it into a high-temperature furnace at 800 °C +/- 25 °C and burn it for 45 min.

When the temperature of the furnace drops to 200 °C, remove the cover, put it in the desiccator to cool down for 30 min, then, weigh it.

A.9.4 Result calculation The mass fraction w_3 of burning residue in the specimen shall be calculated in accordance with Formula (A.5).

Where, m_5 ---after burning, the mass of the crucible and the specimen, expressed in (g);

m_3 ---the mass of the crucible, expressed in (g);

m_4 ---the mass of the crucible and the specimen, expressed in (g).