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GB

NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA

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Feed additive -- 2% d-Biotin

饲料添加剂 2% d-生物素

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Feed additive -- 2% d-Biotin

1 Scope

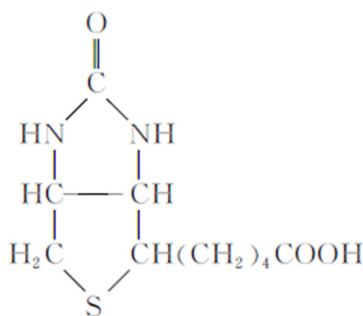
This Standard specifies the requirements, test methods, inspection rules and requirements for labeling, packaging, transportation and storage of feed additive 2% d-Biotin products.

This Standard is applicable to feed additives containing 2% d-Biotin prepared by spraying method and dilution method with starch, dextrin or lactose as carriers.

Molecular formula: $C_{10}H_{16}N_2O_3S$.

Relative molecular mass: 244.31 (2005 international relative atomic mass).

Structural formula:



2 Normative references

The provisions in following documents become the provisions of this Standard through reference in this Standard. For dated references, the subsequent amendments (excluding corrigendum) or revisions do not apply to this Standard, however, parties who reach an agreement based on this Standard are encouraged to study if the latest versions of these documents are applicable. For undated references, the latest edition of the referenced document applies.

GB/T 602, *Chemical reagent - Preparations of stock standard solutions* (GB/T 602-2002, ISO 6353-1:1982, NEQ)

GB/T 603, *Chemical reagent - Preparations of reagent solutions for use in test methods* (GB/T 603-2002, ISO 6353-1:1982, NEQ)

GB/T 6435, *Determination of moisture in feedstuffs* (GB/T 6435-2006, ISO

Pipette 0.50mL of trifluoroacetic acid into a 1000mL volumetric flask. Use ultrapure water to set volume constant to the scale mark.

4.1.5 Extractant: trifluoroacetic acid solution (4.1.4) + acetonitrile (4.1.1) = 75 + 25.

4.1.6 d-Biotin reference substance: d-Biotin content $\geq 99.0\%$.

4.1.7 d-Biotin standard stock solution: Weigh about 100mg of (accurate to 0.00001g) biotin reference substance (4.1.6). Place in a 50mL volumetric flask. Use extractant (4.1.5) to dissolve. Dilute and set volume constant to the scale mark. Shake well. The standard stock solution contains 2.0mg of biotin per milliliter.

4.1.8 d-Biotin standard working solution: Accurately pipette 1.00mL of d-Biotin standard stock solution (4.1.7) into a 10mL volumetric flask. Use extractant (4.1.5) to dilute and set volume constant to the scale mark. Shake well. The standard working solution contains 200 μ g of biotin per milliliter.

4.2 Instruments and equipment

Common laboratory equipment and:

4.2.1 Ultrasonic water bath.

4.2.2 Ultrapure water device.

4.2.3 High-performance liquid chromatograph, with a UV tunable wavelength detector (or diode matrix detector).

4.3 Identification test

Take the specimen solution. Measure it by high performance liquid chromatography. The relative retention time of the main peak of the sample solution is consistent with the relative retention time of the main peak of the control solution.

4.3.1 Preparation of test solution

Weigh about 0.5g of the specimen (accurate to 0.0002g). Put in a 50mL volumetric flask. Add about 40mL of extractant (4.1.5). Conduct ultrasonic extraction in an ultrasonic water bath for 15min. Cool to room temperature. Use extractant (4.1.5) to set volume constant to the scale mark. Shake well. Filter. The filtrate passes through a 0.45 μ m filter membrane for analysis by high performance liquid chromatography.

4.3.2 Chromatographic conditions

Stationary phase: C₁₈ column; inner diameter is 4.6mm; length is 250mm; particle size is 3 μ m.

Mobile phase: trifluoroacetic acid solution (4.1.4) + acetonitrile (4.1.1) = 75 + 25.

50 - The dilution ratio of the test solution (4.3.1);

P_{st} - The peak area of d-Biotin standard working solution (4.1.8);

m - The mass of the specimen, in grams (g).

4.4.2.3.2 Parallel determination results are expressed by arithmetic mean. Reserve three significant figures.

4.4.2.4 Repeatability

The relative deviation of the results of two simultaneous parallel determinations of the same specimen by the same analyst shall not be greater than 5.0%.

4.5 Determination of loss on drying

Determine according to GB/T 6435.

4.6 Determination of arsenic

4.6.1 Weigh 1g of the specimen (accurate to 0.0001g) into a 30.0mL porcelain crucible. Add 5mL of 150g/L magnesium nitrate solution. Mix well. Evaporate to dryness at low temperature or in a boiling water bath. After low-temperature carbonization until smokeless, transfer to a high-temperature furnace for constant-temperature ashing at 550°C for 3.5h~4.0h. Take out and cool. Slowly add 10.0mL of hydrochloric acid solution (4.1.3). After violent reaction, boil and transfer to a generator. Add 8.0mL of hydrochloric acid. Add water to about 40.0mL.

Accurately pipette 3mL of 1.0μg/mL arsenic standard working solution into the generator. Add 10mL of hydrochloric acid. Add water to dilute to 40mL. Add potassium iodide solution.

4.6.2 The following is determined according to Method One Arsenic salt inspection method (ancient Cai's method) in *The Pharmacopoeia of the People's Republic of China (2005 Edition, Volume Two)*.

4.7 Determination of heavy metals

4.7.1 Weigh 1g of the specimen (accurate to 0.001g) into a 30mL porcelain crucible. After low-temperature carbonization until smokeless, transfer to a high-temperature furnace for constant-temperature ashing at 550°C for 3.5h~4.0h. Take out and cool. Slowly add 10mL of water. Boil, filter and transfer to a colorimetric tube. Rinse with a small amount of water several times. Set the volume to 25.0mL.

4.7.2 The following is determined according to Method Three Heavy metal inspection method in *The Pharmacopoeia of the People's Republic of China (2005 Edition, Volume Two)*.

4.8 Granularity

Weigh 50.0g of specimen. Use a shaking sieve. After 5min, the mass of the specimen remaining on the 0.18mm aperture (80 mesh) analytical sieve shall not exceed 2.5g.

5 Inspection rules

5.1 Exit-factory inspection

The feed additive 2% d-Biotin shall be inspected by the quality supervision department of the manufacturer according to this Standard. All indicators specified in this Standard are exit-factory inspection items. Manufacturers shall ensure that all d-Biotin products meet the requirements specified in this Standard. Each batch of products can only leave the factory after passing the inspection.

5.2 Sampling method

Clean, dry, airtight sample bottles are required for sampling. A label is attached to the bottle and marked with manufacturer name, product name, batch number, sampling date.

When sampling, use a clean and suitable sampling tool to insert three-quarters of the depth of the material layer. Mix the samples taken thoroughly. Use quartering method to reduce. Each batch of samples is divided into two portions. The amount of each portion shall be three times the amount of the specimen required for inspection. Load into the sample bottles. One bottle is for inspection. The other bottle is sealed, stored for possible inspection.

5.3 Rules for determination

If one of the indicators in the inspection results does not meet the requirements of this Standard, the sampling shall be doubled for re-inspection. If there is still one indicator in the re-inspection results that does not meet the requirements of this Standard, the entire batch of products shall be rejected.

6 Labeling, packaging, transportation and storage

6.1 Labeling

According to GB 10648.

6.2 Packaging

Products are accurately weighed and packed into aluminum foil bags or metal cans. Seal. Store in outer packaging containers. Store airtight.

6.3 Transportation

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