GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

ICS 65.120

B 46

GB/T 17778-2005

Replacing GB/T 17778-1999

Determination of d-biotin in premix

预混合饲料中 d-生物素的测定

Issued on: September 05, 2005 **Implemented on: February 01, 2006**

Issued by: General Administration of Quality Supervision, Inspection and Quarantine of PRC;

Standardization Administration of PRC.

Table of Contents

Determination of d-biotin in premix

1 Scope

This standard specifies two methods for the determination of d-biotin content in premixed feed, by spectrophotometer and high performance liquid chromatography.

Method 1 and Method 2, as specified in this standard, are applicable to the determination of compound premix feed and vitamin premix feed, which has a d-biotin content greater than 1.0 mg/kg. The concentration range of the extract is 2.0 μg/mL ~ 20.0 μg/mL.

2 Normative references

The provisions in following documents become the provisions of this Standard through reference in this Standard. For the dated references, the subsequent amendments (excluding corrections) 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 6682 Water for analytical laboratory use - Specification and test methods (neq ISO 3696:1987)

GB/T 14699.1 Feeding stuffs - Sampling

3 Method 1 - Spectrophotometry

3.1 Principle

Extract the d-biotin in the specimen, through the ethanol solution. The d-biotin and 4-dimethylaminocinnamaldehyde in the sulfuric acid ethanol solution will form an orange compound. The color depth, within a certain range, is directly proportional to the d-biotin content.

3.2 Reagents and materials

Unless otherwise stated, only reagents of confirmed analytical grade are used in the analysis. The water complies with the provisions of grade-3 water in GB/T 6682.

3.2.1 Absolute ethanol.

3.2.2 Ethanol solution: Mix 90 volumes of ethanol with 10 volumes of water.

3.2.3 Sulfuric acid-ethanol solution: Mix 2 volumes of sulfuric acid with 98 volumes of absolute ethanol.

3.2.4 4-Dimethylaminocinnamaldehyde absolute ethanol solution: 2 g/L.

3.2.5 d-biotin standard solution

3.2.5.1 Standard stock solution: Accurately weigh 0.1000 g of d-biotin standard substance. Dissolve it in ethanol solution (3.2.2). Quantitatively transfer it to a 100 mL volumetric flask. Use ethanol water solution to dilute to the mark. Mix well.1.00 mL of this solution contains 1.00 mg of d-biotin.

3.2.5.2 Standard working solution: Accurately pipette 1.00 mL of d-biotin standard stock solution (3.2.5.1) into a 50 mL volumetric flask. Add ethanol solution, to dilute to the mark. Mix well.1.00 mL of this solution contains 20.0 μg of d-biotin,

3.3 Instruments and equipment

3.3.1 Spectrophotometer (with one derivative function).

3.3.2 Ultrasonic extractor for laboratory use.

3.3.3 Analytical balance: Sensitivity 0.0001 g.

3.4 Preparation of specimens

Carry out sampling according to the method specified in GB/T 14699.1. Select a feed sample of at least 500 g. Reduce it to 100 g by quartering. Grind it. Pass it through a 0.42 mm hole sieve. Mix it. Put it in an airtight container. Save it for later use.

3.5 Analytical procedures

3.5.1 Extraction of specimen solution

Weigh about 2 g of vitamin premix (accurate to 0.0001 g) AND about 5 g ~ 10 g of compound premix (accurate to 0.000 1 g). Put them in a ground-bottomed flat-bottomed flask. Add 5.00 mL of water to an ultrasonic extractor (3.3.2) for 20 min. Add 20 mL of absolute ethanol (3.2.1). Sonicate it for 20 min. Then transfer to a 50 mL volumetric flask. Use absolute ethanol (3.2.1) to dilute it to the mark. Filter it. Discard the first 10 mL. Use the rest as specimen extract.

3.5.2 Determination

3.5.2.1 Drawing of standard working curve

Accurately pipette 0.00, 1.00, 2,00, 5.00, 10.00 mL of d-biotin standard working

solution (3.2.5.2), into a 25 mL volumetric flask. Add 10.00, 9.00, 8.00, 5.00, 0.00 mL of ethanol aqueous solution (3.2.2), respectively. Add 1 mL of sulfuric acid-ethanol solution (3.2.3) and 2 mL of 4-dimethylaminocinnamaldehyde dehydrated ethanol solution (3.2.4). Shake well. Place at room temperature for 1 h. Use absolute ethanol solution (3.2.1) to dilute it to the mark. Use a 1.0 cm cuvette, to scan the first derivative of absorbance by a spectrophotometer, at 500 nm ~ 580 nm. Draw the standard working curve of the d-biotin content and the peak difference of the first derivative of absorbance, at 520 nm and 546 nm.

3.5.2.2 Accurately pipette 10.00 mL of 3.5.1 specimen extract, into a 25 mL volumetric flask. Add 1 mL of sulfuric acid-ethanol solution (3.2.3) and 2 mL of 4-dimethylaminocinnamon yeast absolute ethanol solution (3.2.4). Shake well. Let it stand at room temperature for 1 h. Use absolute ethanol (3.2.1) to dilute it to the mark. Use a 1.0 cm cuvette at 500 nm ~ 580 nm, to measure the peak difference of the first derivative of absorbance at 520 nm and 546 nm, through a spectrophotometer. Find the d-biotin content in the specimen extract, from the standard working curve.

3.6 Calculation and presentation of analysis results

3.6.1 The content of d-biotin in the specimen is calculated according to formula (1).

$$X = \frac{m_1 V_2}{m_2 V_1} \qquad \cdots\cdots (1)$$

Where:

X - The content of d-biotin in the specimen, in milligrams per kilogram (mg/kg);

m_1 - The mass of d-biotin in the specimen extract, as obtained from the standard curve, in micrograms (μg);

m_2 - The specimen mass, in grams (g);

V_1 - The volume of the specimen extraction solution, which is pipetted during specimen determination, in milliliters (mL);

V_2 - The total volume of the specimen extract, in milliliters (mL).

3.6.2 For each specimen, take two sets of samples for parallel measurement. Take the arithmetic mean as the measurement result. Keep three significant figures.

3.7 Precision

3.7.1 Repeatability

The relative deviation of the two independent test results, which are obtained under repeatability conditions, is specified in Table 1, on the premise that the relative deviation greater than the specified does not exceed 5%.

standard stock solution in a 50 mL volumetric flask. Use water to dilute it to the mark.1.00 mL of this solution contains 20.0 μg of d-biotin.

4.3 Instruments and equipment

4.3.1 Ultrasonic extractor for laboratory use.

4.3.2 High-performance liquid chromatography, which is equipped with ultraviolet or diode matrix detectors.

4.4 Specimen preparation

Same as spectrophotometry 3.4.

4.5 Analytical procedures

4.5.1 Extraction of specimen solution

Weigh about 2 g of vitamin premixed feed (accurate to 0.0001 g) AND about 5 g of compound premixed feed (accurate to 0.0001 g). Place them in a 100 mL volumetric flask (if the premixed feed contains minerals, add 0.1 g of DTPA). Add two-thirds of the volume of distilled water. Ultrasonically extract it in the ultrasonic extractor (4.3.1) for 20 min. After cooling, use water to make its volume reach to the mark. Filter it. Make the filtrate pass through a 0.45 μm filter membrane. Prepare for use.

4.5.2 Determination

4.5.2.1 High performance liquid chromatography conditions

Chromatographic column: A C_{18} column, which has a length of 250 mm, an inner diameter of 4.6 mm, a particle size of 5 μm.

Mobile phase: 850 mL trifluoroacetic acid solution (4.2.2) plus 150 mL acetonitrile (chromatographically pure).

Mobile phase flow rate: 1.0 mL/min.

Injection volume: 20 μL.

Detector: UV or diode matrix detector, which uses a wavelength of 210 nm.

4.5.2.2 Quantitative determination

Adjust the operating parameters of the instrument, according to the instructions of the high-performance liquid chromatograph. Inject the standard working solution (4.2.3.2) and the specimen extraction solution (4.5.1), into the chromatographic column, to obtain the response value of the chromatographic peak area. Take the average value of the peak area of the standard solution, for quantitative calculation.

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