ICS 65.120
CCS B 46

GB

national standards of People's Republic of China

GB 18970—202x

Replace GB/T 18970-2003

Feed Additive Part 9: Coloring Agent β,β - carotene - 4,4 - dione (Cantharidin yellow)

Feed additives —Part 9: Coloring agents–4,4′-diketo-β-carotene (canthaxanthin)

(Draft for comments)

20xx-xx-xx released

20xx-xx-xx implementation

State Administration for Market Regulation

release

National Standardization Management Committee

before Speak

GB 7300 "Feed Additives" is divided into several parts according to products:

This document is part 902 of GB 7300.

This document was drafted in accordance with the rules given in GB/T1.1-2020.

This document replaces GB/T 18970-2003 "Feed additive 10% β, β-carrot-4, 4-dione (10% cantharidin yellow)", and GB/T
Compared with 18970-2003, except for editorial changes, the main changes are as follows:

——Added the feed additive 96% β, β-carotene or vitamin A acetate as the main raw material through chemical synthesis.
Carotene-4, 4-dione products, and all related content;

——The description of the trait is revised to "10% of the product is red to purple-red fluid powdery particles, sensitive to oxygen, heat and light" (see
3.1, 3.1 of the 2003 version);

——The requirement for the analysis sieve with a particle size of 0.42mm has been increased (see 3.2 Table 1);

——The indicators of arsenic and chromium have been added (see 3.2 Table 1);

——Modified the content determination method (see 5.4.2, 2003 version 4.4);

——The regulations on packaging materials and shelf life have been added (see 7);

——Informative Appendix A has been added (see Informative Appendix A).

This document was proposed and managed by the Ministry of Agriculture and Rural Affairs of the People's Republic of China.

Drafting organizations of this document: Feed Quality Supervision, Inspection and Testing Center of the Ministry of Agriculture (Jinan), DSM Vitamin (Shanghai) Co., Ltd., Zhejia
Xinhecheng Co., Ltd., Zhejiang Pharmaceutical Co., Ltd., Zhejiang Xinweipu Additive Co., Ltd.

The main drafters of this document:

Feed Additive Part 9: Coloring Agent

β, β-carotene-4,4-dione

(Cantharidin yellow)

1 Scope

This part of GB 7300 specifies the technical requirements, sampling and testing of feed additives β, β-carotene-4, 4-dione (cantharidin yellow)
Inspection methods, inspection rules, labels, packaging, transportation and storage.

This document is applicable to the feed additive 96% β prepared by chemical synthesis with β-carotene or vitamin A acetate as the main raw material.
β-carotene-4, 4-dione (cantharidin yellow) products, and feeds made from this product as a raw material by adding silica and other carriers and spray-dried
Material additives 10% β, β-carotene-4, 4-dione (cantharidin yellow) products.

Molecular formula: $C_{40}H_{52}O_2$

Relative molecular weight: 564.84

Structural formula:

2 Normative references

The contents of the following documents constitute indispensable clauses of this document through normative references in the text. Among them, dated reference documents, Only the version corresponding to that date is applicable to this document; for undated references, the latest version (including all amendments) is applicable to this document file.

GB/T 5917.1 Two-layer sieve sieving method for feed crushing particle size determination

GB/T 6682 Analytical laboratory water specifications and test methods

GB 10648 Feed label

GB/T 13079 Determination of total arsenic in feed

GB/T 13080 Method for determination of lead in feed

GB/T 13081 Determination of Mercury in Feed

GB/T 13088 Method for determination of chromium in feed

GB/T 14699.1 Feed sampling

3 Technical requirements

3.1 Appearance and traits

96% of the product is a deep purple-red crystalline powder, easily soluble in acetone, insoluble in water, and sensitive to oxygen, heat and light; 10% of the product is red to purple Red fluid powdery particles, sensitive to oxygen, heat and light.

3.2 Technical indicators

The technical indicators are shown in Table 1.

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Table 1 Technical indicators

project		Specifications and indicators	
		96%	10%
β, β-carotene-4, 4-dione (cantharidin yellow) content (calculated as $C_{40}H_{52}O_2$),%	≥	96	10
Loss on drying,%	≤	0.2	8
grain degree	Pass through an analytical sieve with an aperture of 0.84mm,%	-	100
	Pass through an analytical sieve with an aperture of≤0.42mm,%	-	80
Burning residue,%	≤	0.2	-
Other carotenoids,%	≤	5	-
Triphenylphosphine oxide (TPPO), mg/kg	≤	1000	-
Lead, mg/kg	≤	10	10
Arsenic, mg/kg	≤	3	3
Mercury, mg/kg	≤	1	-
Chromium, mg/kg	≤	-	2

4 sampling

4.1 Batch

A batch of products produced with the same material, the same production process, continuous production or the same shift. 96% of the product batch must not exceed Over 10 tons.

4.2 Sampling

Sampling shall be conducted according to the provisions of GB/T 14699.1.

5 Test method

Unless otherwise specified, only use reagents confirmed to be analytically pure and third-grade water that meets the requirements of GB/T 6682 in the analysis.

5.1 Senses

Take an appropriate amount of the sample and place it in a clean, dry white porcelain dish, and observe its color and shape under natural light.

5.2 Granularity

According to the provisions of GB/T 5917.1.

5.3 Loss on drying

5.3.1 Instruments

5.3.1.1 Analytical balance: the sensitivity is 1 mg.

5.3.1.2 Decompression drying oven: the temperature can be controlled at 40℃±2℃, and the vacuum degree is within 40 kPa ~ 53 kPa.

5.3.1.3 Drying box: the temperature can be controlled at 105℃±2℃.

5.3.1.4 Dryer: an effective desiccant is enclosed.

5.3.2 Test procedure

5.3.2.1 Weigh 1.8g ~ 2.2g (accurate to 0.0001 g) of 96% product sample, and place it in a weighing bottle. Use P_2O_5 as the desiccant.
Dry under reduced pressure at 40°C for 4 hours, cool to room temperature in a desiccator, and weigh.

5.3.2.2 Weigh about 1 g of 10% product sample (accurate to 0.0001 g), and place it in an oven at 105℃ to dry to constant weight.
Inside the bottle, open the cap of the weighing bottle, place it in an oven at 105 ℃, and dry to constant weight.

5.3.3 Test data processing

The loss on drying X_1 in the sample is expressed as a mass percentage, and the value is expressed as a %, which is calculated according to formula (1):

$$X_1 = \frac{m_1 - m_2}{m} \times 100 \quad \text{.....(1)}$$

Where:

m_1 — The mass of the sample plus the weighing bottle before drying, in grams (g);

m_2 — The mass of the dried sample plus the weighing bottle, in grams (g);

m — The mass of the sample, in grams (g).

5.3.4 Precision

The absolute difference between two independent measurement results obtained under repeatability conditions for 96% of the products shall not exceed 0.1%. 10% of products are i
The absolute difference between the two independent measurement results obtained below is not more than 5% of the arithmetic mean of the two measurement values.

5.4 96% product identification

Measure the absorption value of the cyclohexane solution under **5.4.1.4** . The maximum absorption value should be between the wavelength of 468 nm and 472 nm.
Scanning spectrum, see informative appendix A.

5.5 β, β-carotene-4, 4-dione (cantharidin yellow) content

5.5.1 96% products

5.5.1.1 Principle

Cantharidin yellow has an absorption peak at a wavelength of 470 nm. According to Beer's law, its absorption intensity is proportional to the concentration of the sample.

5.5.1.2 Reagents or materials

5.5.1.2.1 Trichloromethane.

5.5.1.2.2 Cyclohexane.

5.5.1.3 Instruments

5.5.1.3.1 Analytical balance: Sensitivity is 1 mg.

5.5.1.3.2 Visible spectrophotometer: equipped with 1cm cuvette.

5.5.1.4 Test procedure

5.5.1.4.1 Sample extraction

Weigh 50 mg of the sample (accurate to 0.1 mg) in a 100 mL brown volumetric flask, add 10 mL of chloroform to dissolve the sample, and use a ring
Dilute hexane to the mark, mix well, and use it as a test solution.

5.5.1.4.2 Test solution dilution

Accurately pipette 5 mL of the test solution into a 100 mL brown volumetric flask, dilute to the mark with cyclohexane, mix well, and use as the solution to be diluted. quasi-
Make sure to transfer 5 mL of the solution to be diluted into a 50 mL brown volumetric flask, dilute to the mark with cyclohexane, and mix.

5.5.1.4.3 On-machine measurement

Using a 1cm cuvette, with cyclohexane as a blank reference, measure the maximum absorption value A_{max} of the test solution at a wavelength of 470nm .

5.5.1.5 Test data processing

The content of cantharidin yellow in the sample X_2 , expressed in mass fraction (%), calculated according to formula (2):

$$X_2 = \frac{A_{max}}{m_3} \times 20000 \quad \text{.....(2)}$$

Where:

A_{max} — the maximum absorption value measured by the sample solution;

2200 -Standard percent extinction value of cantharidin yellow in the sample ($E^{1\%}_{1cm}$);

20000 -the dilution factor;

m_3 — The mass of the sample, in grams (g).

5.5.1.6 Precision

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The absolute difference between two independent determination results obtained under repeatability conditions shall not exceed 1.0%.

5.5.2 10% product

5.5.2.1 Principle

Cantharidum yellow has an absorption peak between the wavelengths of 470 nm and 476 nm. According to Beer's law, its absorption intensity is proportional to the concentration of

5.5.2.2 Reagents or materials

5.5.2.2.1 Anhydrous ethanol.

5.5.2.2.2 Dichloromethane.

5.5.2.2.3 Cyclohexane.

5.5.2.2.4 Absolute ethanol cyclohexane solution: V+V=1+9.

5.5.2.3 Instruments

5.5.2.3.1 Analytical balance: the sensitivity is 1 mg.

5.5.2.3.2 Centrifuge: speed 4000r/min.

5.5.2.3.3 Ultrasonic extractor: can be heated to 60°C.

5.5.2.3.4 Visible spectrophotometer: equipped with 1cm cuvette.

5.5.2.4 Test procedure

5.5.2.4.1 Sample extraction

Weigh 0.1 g of the sample (accurate to 0.0001 g) into a 100 mL brown volumetric flask, add 5 mL of water, and dissolve it with ultrasound at 60 ℃

Solve for 5 minutes, quickly cool with cooling water, add 50 mL of absolute ethanol, then dilute to the mark with dichloromethane, mix well, and use as a test solution.

5.5.2.4.2 Test solution dilution

After mixing evenly, transfer an appropriate amount of test solution 4000 r/min and centrifuge for 5 min. Accurately pipette 1 mL supernatant test solution into a 50 mL brown volu

Dilute to the mark with absolute ethanol cyclohexane solution.

5.5.2.4.3 On-machine measurement

Using a 1cm cuvette, with cyclohexane as a blank reference, measure the maximum absorption value A_1 of the test solution at a wavelength between 470 nm and 476 nm .

5.5.2.5 Test data processing

The content of cantharidin yellow in the sample X_3, expressed in mass fraction (%), calculated according to formula (3):

$$X_3 = \frac{A_1 \text{X}5000}{m_4 \text{X}2050} \quad \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots (3)$$

Where:

A_1 — the maximum absorption value measured by the sample solution;

2050 -The standard percent extinction value of cantharidin yellow in the sample ($E^{1\%}_{1cm}$);

5000 —dilution multiple;

m_4 — The mass of the sample, in grams (g).

5.5.2.6 Precision

The absolute difference between the two independent measurement results obtained under repeatability conditions is not more than 5% of the arithmetic mean of the two measureme

5.6 Ignition residue

5.6.1 Instruments

5.6.1.1 Analytical balance: the sensitivity is 1 mg.

5.6.1.2 Crucible: ceramic or other materials.

5.6.1.3 High temperature furnace: the temperature can be controlled at 550 ± 50 ℃.

5.6.2 Test procedure

Weigh about 1 g of the sample (accurate to 0.0001 g) in a crucible that is burned to a constant weight, carefully carbonize it on the electric furnace until there is no black smoke, and

Burn in a high-temperature furnace at 550℃±50℃ for 3h ~ 4h, take it out and cool for 1min, put it in a desiccator, and cool to room temperature. Weigh the residue and crucible

The quality of the pot. Repeat burning for another 1h and weigh to constant weight.

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5.6.3 Test data processing

The content of ignition residue in the sample X_4, expressed in mass fraction (%), calculated according to formula (4):

$$X_4 = \frac{m_6 - m_7}{m_5} \times 100 \quad \text{..... (4)}$$

Where:

m_6 — The mass of the sample plus the crucible before firing, in grams (g);

m_7 — The mass of the dried sample plus the crucible, in grams (g);

m_5 — The mass of the sample, in grams (g).

5.7 Other carotenoids

5.7.1 Principle

Cantharidin yellow (trans and cis) and other carotenoids have absorption peaks at a wavelength of 470 nm. The area normalization method is used to calculate the cantharidin yellov The area of other chromatographic peaks other than the miscellaneous peaks introduced by the separation solvent, the proportion of the area of all components is the content of other carot

5.7.2 Reagents or materials

5.7.2.1 Acetonitrile, chromatographically pure.

5.7.2.2 Water, first-class water.

5.7.3 Instruments

5.7.3.1 Liquid chromatograph: equipped with ultraviolet detector.

5.7.4 Test procedure

5.7.4.1 Accurately pipette 5 mL of the test solution under 5.5.1.4 into a 50 mL brown volumetric flask, dilute to the mark with acetonitrile, shake well, A 0.45μm filter membrane was injected into a high performance liquid chromatograph, the chromatogram was recorded, and the retention time of the trans structure in the cantharidin yell

5.7.4.2 Accurately pipette 5 mL of the test solution under 5.5.1.4 into a round bottom flask, reflux in a water bath at 80°C for 1 hour, and transfer after cooling In a 50 mL brown volumetric flask, make the volume up to the mark with acetonitrile, shake well, pass through a 0.45μm filter membrane, inject into the high performance liquid chromat Spectrogram, determine the retention time of the cis structure in the cantharidin yellow solution (after the retention time of the trans structure).

5.7.4.3 Add 10 mL of chloroform to a 100 mL volumetric flask, dilute to the mark with cyclohexane, and shake well. Pipette 5 mL to 50 mL In a volumetric flask, dilute to the mark with acetonitrile, shake well, pass through a 0.45μm filter membrane, inject into the high performance liquid chromatograph, and record the chron Compare the chromatograms to determine the solvent peak and impurity peak.

5.7.4.4 Reference chromatographic conditions

Chromatographic column: C_{18}, 5μm, 250×4.6mm

Mobile phase: acetonitrile+water=95+5

Flow rate: 1mL/min

Detection wavelength: 470nm

Injection volume: 20μL

Running time: 40min.

5.7.5 Test data processing

The content of other carotenoids in the sample X_5, expressed in mass fraction (%), calculated according to formula (5):

$$X_5 = \frac{Cx}{Cx + \mathrm{Vx}} \times 100 \quad \text{..... (5)}$$

Where:

C_x ——The sum of the area of other peaks in the sample except the cantharidin component and solvent peak and the introduced impurity peak;

V_x ——the sum of the area of cantharidin yellow components in the sample;

The determination result is expressed by the arithmetic mean of parallel determination, and the result retains one significant digit.

5.8 Triphenylphosphine oxide (TPPO)

5.8.1 Principle

TPPO was extracted with tetrahydrofuran, determined by liquid chromatography and quantified by external standard method.

5.8.2 Reagents or materials

5.8.2.1 Isopropanol, chromatographically pure.

5.8.2.2 n-hexane, chromatographically pure.

5.8.2.3 Tetrahydrofuran, chromatographically pure.

5.8.2.4 Triphenylphosphine oxide, purity ≥99%.

5.8.3 Equipment

5.8.3.1 Liquid chromatograph: equipped with ultraviolet detector.

5.8.3.2 Chromatographic column: stainless steel column (150×4.6mm), silica-based column Supelcosil Lc-Si, 5μm or similar column.

5.8.4 Test procedure

5.8.4.1 Preparation of standard solution: accurately weigh 10mg of TPPO standard into a 1000mL volumetric flask, dissolve it with tetrahydrofuran and dilute to Scale and mix well. The concentration of this solution is 10 μg/mL. To be tested after 0.45 filter membrane.

5.8.4.2 Sample extraction: accurately weigh 1 g sample (accurate to 0.01mg) into a 100mL volumetric flask, dissolve it with tetrahydrofuran and dilute to volume To the mark, mix well. To be tested after 0.45 filter membrane.

5.8.4.3 Liquid chromatography conditions

Column temperature: 20℃

Mobile phase: isopropanol+n-hexane=1+24

Flow rate: 1.5mL/min

Injection volume: 50μL

Detection wavelength: 210nm

TPPO retention time: about 8.1min

5.8.5 Test data processing

The content of TPPO in the sample X_6, expressed in milligrams per kilogram (mg/kg), calculated according to formula (6):

$$X_6 = \frac{A_S \, X C_i \, X100}{A_{st} \, X m_8} \quad \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots (6)$$

Where:

C_i ——The concentration of TPPO in the standard sample, in micrograms per milliliter (μg/mL);

A_s ——the chromatographic peak area of TPPO in the sample;

A_{st} ——the chromatographic peak area of TPPO in the standard sample;

m_8 ——The mass of the sample, in grams (g).

The determination result is expressed by the arithmetic mean of parallel determination, and the result retains one significant digit.

5.9 Lead

According to the provisions of GB/T 13080.

5.10 Arsenic

According to the provisions of GB/T 13079.

5.11 Chromium

According to the provisions of GB/T 13088.

5.12 Mercury

According to the provisions of GB/T 13081.

6 Inspection rules

6.1 Factory inspection

Each batch of products should be inspected by the quality inspection department of the manufacturer in accordance with this standard. This standard specifies that all items are facto The manufacturer shall ensure that all manufactured products meet the requirements of this standard. Each batch of products shipped should be accompanied by product quality inspection certificate.

6.2 Type inspection

Type inspection items are all the requirements of Chapter 3. When the product is in normal production, the type inspection shall be carried out at least once every six months, but the In the first case, type inspection is also carried out:

a) When a new product is put into production;

b) When there are major changes in raw materials, equipment, and processing techniques;

c) When the product has been suspended for more than 3 months, when production resumes;

d) When there is a big difference between the factory inspection result and the last type inspection result;

e) When the feed management department requests for type inspection.

6.3 Judgment rules

6.3.1 The test results of the inspected items are consistent with the indicators specified in this standard, and they are judged to be qualified products.

6.3.2 If an indicator in the inspection result does not meet the requirements of this standard, it shall be re-inspected by sampling from twice the amount of packaging. If Fu If the inspection result still does not meet the requirements of this standard, the batch of products shall be deemed unqualified and shall not be shipped.

6.3.3 The limit value in each technical index adopts the rounding value comparison method.

7 Labeling, packaging, transportation and storage

7.1 Label

According to GB 10648.

7.2 Packaging

Use light-proof evacuated or filled with inert gas containers, sealed.

7.3 Transportation

During transportation, it should be protected from light, moisture, high temperature, and rain, and must not be mixed with toxic and harmful substances.

7.4 Storage

96% of the products are stored in a cool, dry place, not mixed with toxic and hazardous substances. 10% of the products are stored in a ventilated, dry, and high-temperature place, Do not mix with toxic and hazardous substances. Under the specified transportation and storage conditions, the shelf life of 96% of the products in the original packaging is 12 months. The shelf life of 10% of the products in the original packaging is 18 months.

Appendix A

(Informative appendix)

Cantharidin yellow UV scanning spectrum

Figure A.1 shows the UV scan spectrum of cantharidin yellow

Figure A.1 Ultraviolet scanning spectrum of cantharidin yellow

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