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National Food Safety Standard - Food Additives - BIXIN

食品安全国家标准 食品添加剂 胭脂树橙

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National Food Safety Standard - Food Additives - BIXIN

1 Scope

This Standard is applicable to the food additive BIXIN (bixin) obtained by taking the outer seed coat of annato (*Bixa orellana* L.) seeds as raw materials, and through extracting a solvent or cold weak alkaline aqueous solution and refining; as well as the food additive BIXIN (norbixin) obtained through hydrolysis of the hot alkaline aqueous solution and refining. The used solvents include acetone, methanol, ethanol, n-hexane, isopropanol, ethyl acetate, basic ethanol and/or carbon dioxide.

2 Molecular Formula, Structural Formula, and Relative Molecular Mass of Iconic Components

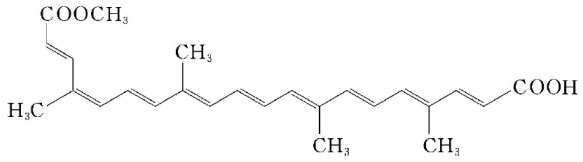
2.1 Molecular formula

Bixin: $C_{25}H_{30}O_4$

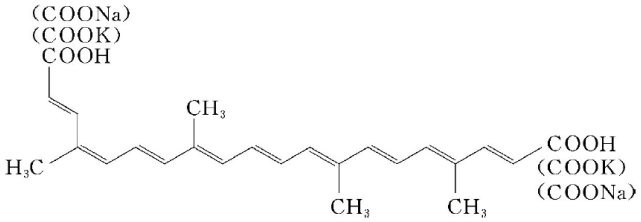
Norbixin: $C_{24}H_{28}O_4$

2.2 Structural formula

Bixin



Norbixin



2.3 Relative molecular mass

Bixin: 394.51 (as per 2018 international relative atomic mass)

Norbixin: 380.48 (by R_1=H, R_2=H) (as per 2018 international relative atomic mass)

3 Classification of Product

3.1 BIXIN (bixin)

3.1.1 BIXIN (bixin extracted by the solvent)

The product that takes the outer seed coat of annato (*Bixa orellana* L.) seed as the raw material, and obtains through solvent extraction and refining (acidification precipitation, solvent removal, drying and crushing).

3.1.2 Bixin (bixin extracted by the alkaline aqueous solution)

The product that takes the outer seed coat of annato (*Bixa orellana* L.) seed as the raw materials, and obtains through being extracted by the cold weak alkaline aqueous solution, and refining (acidification precipitation, solvent removal, drying and crushing).

3.2 BIXIN (norbixin)

3.2.1 BIXIN (norbixin extracted by solvent)

The product that takes the outer seed coat of annato (*Bixa orellana* L.) seed as the raw materials, and obtains through the solvent extraction, refining (solvent removal, crystallisation and drying), then hydrolysing through heating the alkaline aqueous solution, and refining (cooling, filtering, drying, and crushing).

3.2.2 BIXIN (norbixin extracted by alkaline aqueous solution-acidification precipitation-free)

The product that takes the BIXIN (bixin extracted by alkaline aqueous solution) as raw materials, and obtains through hydrolysing the hot alkaline aqueous solution, and refining (cooling, filtering, drying, and crushing).

3.2.3 BIXIN (norbixin extracted by alkaline aqueous solution-acidification precipitation)

The product that takes the BIXIN (bixin extracted by alkaline aqueous solution) as raw materials, and obtained through hydrolysing the hot alkaline aqueous solution, and refining (cooling, filtering, acidification precipitation, drying and crushing).

Appendix A

Inspection Methods

A.1 General provisions

If no other requirements are specified, all the reagents and water used in this Standard refer to the analytical reagents and Class-III water specified in GB/T 6682. If no other requirements are specified, all the standard solution used in the test, standard solution for determining the impurity, preparation and product shall be prepared according to the provisions of GB/T 601, GB/T 602, GB/T 603.The solution used in the test refers to the aqueous solution when the solvent is not specified.

A.2 Identification test

A.2.1 Solubility

BIXIN (bixin) is insoluble in water and slightly soluble in ethanol. Annatto (norbixin) is soluble in alkaline water and slightly soluble in ethanol.

A.2.2 Maximum absorption wavelength

A.2.2.1 BIXIN (bixin)

After BIXIN (bixin) was diluted by acetone, the solution showed three maximum absorption peaks at wavelengths of about 425nm, 457nm and 487nm.

A.2.2.2 BIXIN (Norbixin)

After BIXIN (norbixin) was diluted by potassium hydroxide solution (5g/L), the solution showed two maximum absorption peaks at wavelengths of about 453nm and 482nm.

A.2.3 Thin layer chromatography

Activate the TLC plate (thickness of the thin layer: 250μm; specification: 5cm×20cm) at 110°C for 1h. Prepare a specimen solution with a concentration of 5% (solvent is 95% ethanol); spot 10μL; after drying it, expand by a mixture of n-butanol, methyl ethyl ketone, and 10% ammonia (volume ratio 3: 2: 2) until the front of the solution raises about 10cm and dry it. BIXIN (bixin) and BIXIN (Norbixin) respectively showed yellow spots at the R_f value of about 0.45 ~ 0.50. After spraying by 5% sodium nitrite solution and 0.5mol/L sulfuric acid successively, the yellow spots faded immediately.

A.3 Determination of bixin content

A.3.1 Reagents and materials

A.5.1.3 Internal standard substance: 3-methyl-2-pentanone.

A.5.1.4 Methanol: chromatographically pure.

A.5.1.5 Water: Class-I water specified in GB/T 6682-2008.

A.5.2 Apparatus

Gas chromatograph equipped with hydrogen flame ionization detector (FID) and headspace sampler.

A.5.3 Reference chromatographic conditions

A.5.3.1 Chromatographic column: Quartz capillary column taking 6% cyanopropyl phenyl and 94% dimethyl polysiloxane (or 100% dimethyl polysiloxane) as a stationary phase, with length 60m, inner diameter of 0.53mm, and the coating thickness of 5μm, or a chromatographic column with equivalent performance.

A.5.3.2 Carrier gas: helium.

A.5.3.3 Flow rate: 208kPa, 5mL/min.

A.5.3.4 Column temperature: at 35°C for 5min; then raise to 90°C at a rate of 5°C/min; keep for 19min.

A.5.3.5 Injection port temperature: 140°C.

A.5.3.6 Detector temperature: 300°C.

A.5.4 Reference headspace conditions

A.5.4.1 Sample heating temperature: 60°C.

A.5.4.2 Sample heating time: 10min.

A.5.4.3 Injector temperature: 70°C.

A.5.4.4 Transmission temperature: 80°C.

A.5.4.5 Specimen gas injection: split mode, 1.0mL.

A.5.5 Analysis procedures

A.5.5.1 Method-I (determined in water)

A.5.5.1.1 Preparation of internal standard solution

Pipette 50mL of water into a 50mL injection bottle; seal it; accurately weigh, accurate to 0.0001g. Pipette 15μL of 3-methyl-2-pentanone; inject it into the injection bottle

Heat at 60°C for 10min; shake vigorously for 10s.

A.5.5.2.4 Preparation of specimen solution

Accurately weigh 0.20g of specimen into the injection bottle; then add 5.0mL of methanol and 1.0mL of internal standard solution; heat it at 60°C for 10min; and shake vigorously for 10s.

A.5.6 Determination

Under reference operating conditions in A.5.3 and A.5.4, chromatographically analyse the sample solution, blank solution, and calibration solution. Refer to Figure B.1 in Appendix B for the solvent residue chromatogram.

A.5.7 Calculation of results

A.5.7.1 Calibration factor f_i

The calibration factor f_i of Method-I is calculated according to Formula (A.3).

$$f_i = \frac{m_3 \times 50}{m_4 \times (C - D)} \qquad \cdots\cdots (A.3)$$

Where:

m_3 – mass of standard product of component to be tested in the standard solution, in mg;

50 – conversion factor of mass;

m_4 – mass of the internal standard substance in the internal standard solution, in mg;

C - the ratio of the peak area of the component to be tested to the peak area of the internal standard substance in the standard solution chromatogram;

D - the ratio of the peak area of the component to be tested to the peak area of the internal standard substance in the blank solution chromatogram.

The calibration factor f_i in Method-II is calculated according to Formula (A.4).

$$f_i = \frac{m_3}{m_4 \times (C - D) \times 10} \qquad \cdots\cdots (A.4)$$

Where:

m_3 – mass of standard product of component to be tested in the standard solution, in mg;

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