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# NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB 1886.317-2021

# National Food Safety Standard – Food Additives - β-Carotene (Sourced from Dunaliella)

食品安全国家标准 食品添加剂  $\beta$ -胡萝卜素 (盐藻来源)

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# National Food Safety Standard – Food Additives - β-Carotene (Sourced from Dunaliella)

## 1 Scope

This Standard is applicable to take the *Dunaliella Salina* as the raw materials, and obtain the food additives of  $\beta$ -Carotene (sourced from Dunaliella) through extracting and refining.

# 2 Chemical Name, Molecular Formula, Structural Formula, and Relative Molecular Mass

#### 2.1 Chemical name

1,1'-(3,7,12,16-tetramethyl-1,3,5,7,9,11,13,15,17-octadecenone-1,18-diyl) double [2,6,6-trimethylcyclohexene]

#### 2.2 Molecular formula

 $C_{40}H_{56}$ 

#### 2.3 Structural formula

#### 2.4 Relative molecular mass

536.89 (as per 2018 international relative atomic mass)

## 3 Technical Requirements

#### 3.1 Sensory requirements

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

## 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 Maximum absorption wavelength

Measured by a spectrophotometer, the specimen's cyclohexane solution (5mg/L) has maximum absorption values at 448nm~457nm and 474nm~486nm.

#### A.2.2 Colour reaction

Drop the specimen toluene solution (about 400µg/mL) on the filter paper; spray by 20% antimony trichloride toluene solution; and it turns blue after 2min ~ 3min.

#### A.3 Determination of the total natural carotenoids content

#### A.3.1 Principle of the method

 $\beta$ -carotene is a compound with conjugated double bonds. It has a maximum absorption value at a wavelength of 455nm±1nm, which can be used to determine the total carotenoid content. The specimen is dissolved in chloroform and diluted with hexane to an appropriate concentration. The final dilution is carried out in cyclohexane. The absorbance is measured and the concentration is calculated by the absorbance coefficient.

#### A.3.2 Reagents and materials

A.3.2.1 Chloroform.

A.3.2.2 Cyclohexane.

**A.3.2.3** Hexane.

#### A.3.3 Apparatus

#### A.4.4 Analysis procedures

#### A.4.4.1 Preparation of test solution

#### A.4.4.1.1 $\gamma$ -terpinene internal standard stock solution

Accurately weigh 1.0g of  $\gamma$ -terpinene standard product, accurate to 0.0001g; dissolve in n-hexane; transfer it into a 100mL volumetric flask; use n-hexane to make constant volume to the mark; shake well for later-use.

#### A.4.4.1.2 d-limonene standard stock solution

Accurately weigh 0.5g (accurate to 0.0001g) of d-limonene standard product; dissolve in n-hexane; transfer to a 50mL volumetric flask; use n-hexane to make constant volume to the mark; shake well for later-use.

#### A.4.4.1.3 d-limonene standard working solution

Accurately pipette 5.00mL of d-limonene standard stock solution into a 50mL volumetric flask; use n-hexane to make constant volume to the mark; shake well for later-use.

#### A.4.4.1.4 Serial standard working solution

Accurately pipette 1.00mL of  $\gamma$  -terpinene internal standard stock solution and 10.00mL of d-limonene standard stock solution into a 25mL volumetric flask; dilute to the mark with n-hexane; shake well for later-use. Accurately pipette 2.00mL of  $\gamma$  -terpinene internal standard stock solution and 5.00mL of d-limonene standard stock solution in a 50mL volumetric flask; dilute to the mark with n-hexane; shake well for later-use. Accurately pipette 2.00mL of  $\gamma$  -terpinene internal standard stock solution and 5.00mL of d-limonene standard working solution in a 50mL volumetric flask; dilute to the mark with n-hexane; shake well for later-use. Accurately pipette 2.00mL  $\gamma$  -terpinene internal standard stock solution and 2.00mL of d-limonene standard working solution into a 50mL volumetric flask; dilute to the mark with n-hexane; shake well for later-use. Blank solution, accurately pipette 2.00mL of  $\gamma$  -terpinene internal standard stock solution into a 50mL volumetric flask; dilute to the mark with n-hexane; shake well for later-use. Prepared into a serial standard working solutions containing 0 mg/mL, 0.04 mg/mL, 0.1 mg/mL, 1.0 mg/mL, 4.0 mg/mL of d-limonene and 0.4 mg/mL  $\gamma$  -terpinene, respectively.

#### A.4.4.2 System stability test

**A.4.4.2.1** When the gas chromatograph operates the blank solution for twice, there shall be no d-limonene chromatographic peak.

**A.4.4.2.2** When the gas chromatograph operates the serial standard working solution

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