



**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB 1886.172-2016

**National Food Safety Standard -- Food Additives -- Rosemary
Extract**

食品安全国家标准 食品添加剂 迷迭香提取物

(English Translation)

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1 Scope

This standard applies to the stem rosemary (*Rosmarinus officinalis* L.), Leaves as raw material by solvent extraction or supercritical carbon dioxide Extraction, refining process to produce food additives rosemary extract. The extraction solvent is water, methanol, ethanol, acetone, and (or) hexane.

2.1 Sensory requirements

Sensory requirements shall comply with the requirements in Table 1.

Table 1 Sensory requirements

Project requires test methods Powder or a liquid state Proper amount of sample is placed in a clean, dry beaker or porcelain dish, from the Then observe the state of light

2.2 Physical and Chemical Indicators

Physical and chemical indicators should be consistent with the provisions of Table 2.

Table 2. Physical and chemical indicators

project

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Fat-soluble water-soluble Testing method the total antioxidant component (in terms carnosic acid and carnosol), w /% ≥ 10.0 - Appendix A A.2 Rosmarinic acid, w /% ≥ 5.0 Appendix A A.3 Moisture a, w /% ≤ 5.0 GB 5009.3 distillation or Karl Fischer method Lead (Pb)/(mg/kg) ≤ 2.0 GB 5009.75 or GB 5009.12 Arsenic (As)/(mg/kg) ≤ 3.0 GB 5009.76 Residual solvent b Hexane/(mg/kg) $\leq$ Methanol/(mg/kg) $\leq$ Appendix A A.4 for only a water-soluble powder products.

b only for the extraction solvent is n-hexane or methanol product.

NOTE: commercial rosemary extract product should comply with the standard rosemary extract as a raw material may be added for the processing, storage, standardization, dissolution Process

purpose edible vegetable oils, maltodextrin, sodium chloride and other food materials and (or) meet the requirements of Food Additive Specifications emulsifiers, anti-caking Agent.

Appendix A

Testing method

A.1 General Provisions at time of the reagents in water and does not indicate other requirements, refer to three analytical reagent water and GB/T 6682 provisions. Test when the standard solution, the standard solution, the determination of impurities, formulations and articles not indicate other requirements, are by GB/T 601, GB/T 602, GB/T 603 Preparation predetermined. The test does not indicate when the solution which is formulated with a solvent, refer to the aqueous solution.

A.2 total antioxidants (to carnosic acid and carnosol meter) measured A.2.1 Method summary the main antioxidants (carnosol and carnosic acid) of the rosemary extract liposoluble maximum absorption at a wavelength of 280nm Detection Peak received, in accordance with standard concentration of carnosic acid and the corresponding peak area of the standard curve equation can be derived carnosic acid, and a standard curve Sample peak area corresponding to the sample solution to give a concentration. Meanwhile, since at 280 nm, the peak area of the same concentration of carnosol is sage Acid 1.36 times. Thus the concentration can be calculated based on the standard curve carnosol carnosic acid.

A.2.2 Reagents and materials A.2.2.1 Water. in line with a water GB/T 6682 provisions.

A.2.2.2 acetone. chromatography.

A.2.2.3 Acetonitrile. chromatographically pure.

A.2.2.4 carnosic acid standard. purity $\geq 98\%$.

Phosphoric acid solution A.2.2.5. 1 -> 1000.

A.2.3 Instruments and Equipment HPLC. equipped with a UV detector.

A.2.4 Reference chromatographic conditions A.2.4.1 Column. C18 reversed-phase column (phi4.6mm x 250mm, 5 μ m); or other equivalent column.

A.2.4.2 Mobile phase A. aqueous phosphoric acid solution.

A.2.4.3 mobile phase B. acetonitrile phosphoric acid solution.

A.2.4.4 Column temperature. 30 °C.

A.2.4.5 Detection wavelength. 280nm.

A.2.4.6 mobile phase flow rate. 1.0mL/min.

A.2.4.7 Injection volume. 10 μ L.

A.2.4.8 gradient conditions. see Table A.1.

Table A.1 gradient elution

Time/min Mobile phase A /% Mobile Phase B /% flow rate/(mL/min) 0.0 7723 1.0 1.0 7723 1.0 0 100 25.0 1.0 0 100 30.0 1.0 7723 1.0 30.5 7723 1.0 35.0 A.2.5 Analysis step A.2.5.1 standard curve Carnosic acid was dissolved in acetone standard, prepared a mixed standard solution gradient, a concentration gradient that the carnosic acid 0.010mg/mL ~ 1.000mg/mL. In reference A.2.4 chromatographic conditions, the standard solution is measured, a repetitive injections. The concentration of the standard solution and Peak area of the standard curve. Linear relationship should reach $R^2 \geq 0.99$. Recording standard curve linear equation $Y = a \times c + b$. Where, c is the concentration of carnosic acid, Y is the peak area corresponding to the concentration, a and b are the slope and intercept of the standard curve.

Preparation of sample solution A.2.5.2 Sample Weigh 140mg ~ 180mg (accurate to 0.0001g), was dissolved in 20mL acetone and 25mL volumetric flask to volume, mixed well After filtered through a 0.22μm uniform microporous membrane.

A.2.5.3 Determination in reference A.2.4 chromatographic conditions, the sample was subjected to measurement, a repeated injection. Referring to FIG B.1 rosemary extract identify spectrum Carnosic acid and carnosol relative positions of the two peaks determined in response to, and record the peak area of both Y1 and Y2.

A.2.6 calculation results A.2.6.1 carnosic acid concentration c1 Carnosic acid concentration c1, in milligrams per milliliter (mg/mL), calculated according to formula (A.1).

Y1-b

Where.

Peak area of sample solution Y1 --- carnosic acid;

B --- sage standard curve formula intercept acid;

A --- carnosic acid in the slope of the standard curve formula.

A.2.6.2 carnosol concentration c2 Carnosol concentration c2, in milligrams per milliliter (mg/mL), is calculated according to equation (A.2).

Y2-b

1.36a

Where.

Y2 --- sample solution carnosol peak area;

B --- sage standard curve formula intercept acid;

1.36 --- at 280nm, the peak area of the same concentration was 1.36 times carnosol carnosic acid;

A --- carnosic acid in the slope of the standard curve formula.

Fraction of the total mass of the antioxidant A.2.6.3 w1 the total antioxidant component (in terms carnosol and carnosic acid) of mass fraction w1, according to equation (A.3) is calculated.

$c_1 c_2 () \times V$

$m \times 100\%$

Where.

C1 --- sample solution carnosic acid concentration, in milligrams per milliliter (mg/mL);

c2 sage --- phenol concentration in the sample solution, in milligrams per milliliter (mg/mL);

V --- sample constant volume in milliliters (mL);

--- m sample mass, in milligrams (mg).

The test result to the arithmetic mean of replicates results. Obtained in two independent determination results under repeatability conditions of absolute difference Value is not more than 5% of the arithmetic mean.

Determination of rosmarinic acid A.3 A.3.1 Method summary Water-soluble rosemary extract was dissolved in methanol, - phosphoric acid solution as the mobile phase, octadecylsilane filler for liquid chromatography of Column and UV spectrum detector or diode array detector, sample rosmarinic acid RP-HPLC separation and determination, and standard Retention time qualitative comparison of the quasi-peak area external standard.

A.3.2 Reagents and materials A.3.2.1 Methanol. HPLC grade.

A.3.2.2 rosmarinic acid standard. purity $\geq 98\%$.

Phosphoric acid solution A.3.2.3. 0.5mL phosphate was added 100mL of water.

A.3.2.4 standard stock solution. Accurately weigh 10 mg of rosmarinic acid standard (accurate to 0.0001g), dissolved in mobile phase and dilute to 10mL, mixing, home refrigerator. 1mL of this solution containing rosmarinic acid 1.0mg.

A.3.3 Instruments and Equipment HPLC. equipped with UV detector or diode array detector.

A.3.4 Reference chromatographic conditions A.3.4.1 Column. octadecyl silane column filler (phi4.6mm x 250mm, 5 μ m); or other equivalent color Spectrum column.

A.3.4.2 Mobile phase. methanol. phosphoric acid = 45. 55.