National Standard of the People's Republic of China

GB 1886.172-2016

National food safety standard for rosemary extract

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Food safety standard

Food additive rosemary extract

1 Scope

This standard applies to food additive rosemary extract which use rosemary (Rosmarinus officinalis L.) stems, leaves as raw material, made by the supercritical carbon dioxide or organic solvent extraction 、refined and other process. The extraction solvent was water methanol, ethanol, acetone and / or hexane.

2 technical requirement

2.1 Sensory

See requirements in Table 1.

Table 1

Item	Requirements	Inspection method
Form	Liquid or powder	placed sample in white enamel disc , observe the form in natural light

Physical and chemical indicators See requirements in Table 2

Table 2

item		index		Test method
		Oil soluble	water soluble	
Total antioxidant ingredients，w/ % (meter as carnosic acid and carnosol) %	≥	10.0	—	AppendixA.2
Rosmarinic acid/(%) /%	≥	—	5.0	AppendixA.3
Moisture /%[a]	≤	—	5.0	GB 5009.3 Distillation and Carle Fischer method
Pb /（mg/kg）	≤	2		GB 5009.75
As /（mg/kg）	≤	3		GB 5009.76
Solvent residue,[b] hexane/（mg/kg） methanol /（mg/kg）	 ≤ ≤	 25 50		AppendixA.4
[a] only for powder product。				

B only for solvent by hexane or methanol

Note : The commercialization of rosemary extract products can adding edible vegetable oil , maltodextrin, NaCl, and other food material and other food additives which meet the standard of emulsifying agent, anticaking agent so on

Appendix A

Test method

A. 1 general rules

Unless otherwise specified, the purity of reagents used were of analytical grade, the standard titration solution, the determination of impurities in standard solution, preparation and product, according to the provisions of GB/T 601, GB/T 602, GB/T 603 preparation, test water shall meet the requirements of three degree of GB/T 6682-2008. In the test solution used in the preparation of solvent does not indicate which is it ,it will be the aqueous solution

A. 2 Determination of total antioxidant(meter as carnosic acid and carnosol)

A.2.1 method

Rosemary extract major antioxidants (carnosic acid and carnosol) detection at 280 nm wavelength absorption peak, according to the carnosic acid standard products concentration and the corresponding peak area, The standard carnosic acid curve equation can be drawn, and obtain the corresponding sample concentration from the peak area . Meanwhile, since at 280 nm, the same concentration of carnosol and carnosic acid peak area is 1.36:1. Thus the carnosol concentration can be calculated from the standard curve of carnosic acid.

A.2.2 Reagents and materials

A.2.2.1 Water: in line with the provisions of the GB/T6682 level of water..

A.2.2.2 Acetone. chromatographic purity

A.2.2.3 Acetonitrile: chromatographic purity.

A.2.2.4 Carnosic acid standard: the purity of more than 98%.

A.2.2.5 Phosphoric acid solution: 1 to 1000.

A.2.3 Instruments and equipment

High performance liquid chromatography: with a UV detector.

A.2.4 Reference chromatographic conditions

A.2.4.1 Column: C18 reverse phase column (4.6 mm × 250 mm, 5 μm); or other equivalent column.

A.2.4.2 Mobile phase A: water + 0.1% of phosphoric acid;

A.2.4.3 Mobile phase B: acetonitrile + 0.1% of phosphoric acid.

A.2.4.4 Column temperature: 30 ℃.

A.2.4.5 Detection wavelength: 280 nm.

A.2.4.6 Mobile phase flow rate: 1.0 mL / min.

A.2.4.7 Sample size: 10μ L.

A.2.4.8 Gradient elution conditions:

Gradient elution conditions

TIME（min）	Mobile phase A%	Mobile phase B%	FLOW（mL/min）
0.0	77	23	1.0
1.0	77	23	1.0
25.0	0	100	1.0
30.0	0	100	1.0
30.5	77	23	1.0

35.0	77	23	1.0

A.2.4.7 Injection volume: 10 μL.

A.2.5 Analysis steps

A.2.5.1 Standard curve:

Dissolved in acetone carnosic acid standard sample, make a gradient mixed standard solution , so that carnosic acid concentration gradient in 0.010 mg / mL ~ 1.000 mg / mL. In A.2.4 reference chromatographic conditions, the standard solution were measured, repeated injections once. According to the standard concentration and peak area, draw the standard curve. Linear relationship should reach R2≥0.99.

Recording standard curve linear equation Y = a × C + b. Wherein, 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.

A.2.5.2 Preparation of test solution

Weigh the sample 140 mg ~ 180 mg, denoted as m, was dissolved in 20 mL acetone, 25 mL volumetric flask to volume (referred to as V), after sufficiently mixing, filt with 0.22 μm filter.

A.2.5.3 Determination

In A.2.4 Reference chromatographic conditions, the sample solution was measured, repeated injections once. Refer to Appendix B, Figure B.1 rosemary extract high-performance liquid chromatogram relative position carnosic acid and carnosol to determine the response of the two peaks, and record the peak area of both Y1 and Y2.

A.2.6 Calculate

A.2.6.1 The concentration of carnosic acid

C1-- Carnosic acid concentration, in milligrams per milliliter (mg / mL);

$$C_1 = \frac{Y_1 - b}{a} \quad \text{..... (A.1)}$$

Where:

Y1-- Carnosic acid peak area;

a - Carnosic acid standard curve slope

b - Carnosic acid standard curve intercept

A.2.6.2The concentration of Carnosol

C2-- Carnosol concentration, in milligrams per milliliter (mg / mL);

$$C_2 = \frac{Y_2 - b}{1.36a} \quad \text{..... (A.2)}$$

Where:

Y2-- Carnosol peak area;

a - Carnosic acid standard curve slope

b - Carnosic acid standard curve intercept

1.36-- At 280 nm, carnosol, carnosic acid peak area is 1.36.

A.2.6.3 Calculated results

The total antioxidant content (by carnosic acid and carnosol meter) of the mass fraction of W1 (A.3) is calculated according to the formula:

Carnosic acid and carnosol mass fraction X, respectively, according to the formula (A.3) is calculated:

$$w_1 = \frac{(C_1 + C_2) \times V}{m} \times 100\% \quad \text{..... (A.3)}$$

Where:

C1 -- the concentration in the sample solution of carnosic acid, in mg per ml (mg/mL);

C2-- The concentration in the sample solution -- carnosol, in mg per ml (mg/mL);

V-- sample volume, units of milliliters (mL);

M-- sample mass, the unit is milligram (mg).

The experimental result is the result of the arithmetic mean of the parallel determination.

The absolute difference between the results obtained in two independent repeated under the condition of no more than 5 %.

A.3 Rosmarinic acid

A.3.1 Methods

Water soluble rosemary extract dissolved in methanol, methanol and phosphoric acid solution as mobile phase, using ODS as filler in the liquid phase chromatographic column and ultraviolet detector or a diode array det ector of carnosol was described by reversed phase high performance liquid chromatography separation and det ermination, Comparison of the retention time, and the standard for qualitative, quantitative peak area external s tandard method used to.

A.3.2 Reagents and materials

A.3.2.1 Methanol Chromatographic purity.

A.3.2.2: rosemarinic standard: the purity ≥98%.

A.3.2.3 phosphoric acid solution :0.5 mL phosphoric acid phosphoric acid solution with 100mL water.

A.3.2.4 Standard stock solution: weigh accurately rosmarinic acid standard 10 mg (accurate to 0.0001 g), with 2 mL methanol solution and distilled water to volume 10 mL, mixing, stored in a refrigerator. The solution containing 1 mL and 1 mg of carnosol.

A.3.3 Instrument and equipment

High performance liquid chromatography with UV detector, and diode array detector.

A.3.4 Reference the chromatographic conditions

A.3.4.1 Column: the column length 250 mm, diameter 4.6 mm, built-in ODS filler, particle size of 5 μ m. Or other equivalent chromatographic columns

A.3.4.2 Mobile phase: methanol : phosphoric acid solution =45:55;

A.3.4.3 Flow rate: 0.6 mL/min;

A.3.4.4 Column temperature: 40℃;

A.3.4.5 Detection wavelength: 283nm;

A.3.4.6 The retention time: 18 min

A.3.5 Analysis procedure

A.3.5.1 The sample solution preparation

Weigh accurately and uniformly mixing water soluble rosemary extract were 200mg (accurate to 0.0001 g), dissolved in water and set the volume at 100 mL, after 0.45 m of micro filtration membrane, the sample solution.

A.3.5.2 Drawing standard curve

Accurate drawing standard stock solution of 0mg / ml、 0.25mg / ml、 0.5mg / ml、 1.0mg / ml, Chromatographic analysis was carried out under the condition of A.3.4 reference chromatogram, according to the different amount of sample carnosol standard solution and the corresponding peak area, with the chromatographic peak area as the ordinate, the content of rosmarinic acid as the abscissa and draw standard curve.

A.3.5.3 Determination

Accurate sample solution 10 μl, under the conditions of the specified chromatography, chromatographic analysis, to retain the time qualitative, quantitative peak area outside the standard method.

A.3.5.4 Calculation

W2 mass fraction of rosmarinic acid in the sample, according to the formula (A.4) calculation

$$w_2 = \frac{c \times v}{1\,000 \times m} \times 100\% \quad \text{……（A.4）}$$

Where:

C——The concentration of rosmarinic acid obtained from the standard curve of the sample solution in units of nanograms per milliliter,（μg/mL）;

V——sample liquid volume, units of milliliters（mL）;

1000——Mass conversion factor

m—— sample quality, the unit is （mg）。

The experimental results with the results of parallel determination of the arithmetic mean of the quasi. The absolute difference between the results obtained in two independent repeated under the condition of not more than 2.5% of the arithmetic mean value.

A.4 Residual solvents (hexane, methanol).

A.4.1 Reagents and materials

A.4.1.1 Water :first grade of GB/T 6682

A.4.1.2 Sodium Chloride Solution: 10 g sodium chloride dissolved in 100 mL water.

A.4.1.3 component to be measured standard: n-hexane and methanol, chromatographic purity.

A.4.1.4 Internal standard solution, 160 μg/g normal propyl alcohol solution.

A.4.2 Instrument and equipment

GC: Equipped with hydrogen flame ionization detector (FID) and headspace sampler.

A.4.3 Reference the chromatographic conditions

A.4.3.1 Column: quartz capillary column (diameter of 0.15μ m * 15 m), the coating is 6% cyanopropyl phenyl methyl polysiloxane and 94% two, the thickness of the coating is 0.84 μ m. Or equal the performance of the chromatographic column.

A.4.3.2 Carrier gas: helium.

A.4.3.3 Carrier gas flow rate: 0.8 mL/min.

A.4.3.4, Column temperature: 40 ℃ keep 5 min, heated to 250 ℃ in the rate of 25 ℃ /min, the total running time is 13.4min

A.4.3.5 Inlet temperature: 250 ℃

A.4.3.6 Detector temperature: 300 ℃

A.4.3.7 Sample size: 1 mL.

A.4.4 Reference the headspace condition

A.4.4.1 Sample heating temperature: 90 ℃

A.4.4.2 Sample heating time: 10 min.

A.4.4.3 Sample stirring speed: :400r/min.

A.4.4.4 Split ratio: 1:50

A.4.4.5 Injector temperature: 120 ℃

A.4.4.6 Injection speed: 1 mL/s

A.4.5 Analysis procedure

A.4.5.1 Standard solution preparation

The measured standard components (hexane and methanol), were prepared by standard stock solution of 260μ g/g.

A.4.5.2 Series of standard solution preparation

According to table A.2 and sunflower oil, adding standard stock solution, Sodium Chloride Solution and internal standard solution, preparation of standard solution series, were injected 20 mL sample bottle.

Table A.1 The preparation method of standard solution series

Sunflower oil /mg	Standard stock solution /mg	Sodium Chloride Solution /mg	Internal standard solution /mg
250	50	2700	1000
250	100	2650	1000
250	200	2550	1000
250	500	2250	1000
250	1000	1750	1000

A.4.5.3 Sample solution preparation

Take 0.25 g samples (accurate to 0.0001 g), the addition of sodium chloride is 2750 mg and the internal standard solution for 1000 mg, with 20 mL sample bottle.

A.4.5.4 Determination

In the A.4.3 and A.4.4 reference operating conditions, respectively on the series of standard solution and sample solution were analyzed with gas chromatography.

A.4.5.5The results of calculation

A.4.5.6The average response factor f_i

The average response factor standard solution f (A.5) is calculated according to the formula:

$$f = \frac{\sum\left(\frac{C_s}{C_i} \times \frac{R_i}{R_s}\right)}{5} \quad \text{.....（A.5）}$$

Where:

C_s——The concentration of solvent for each standard solution, units of micrograms per gram（μg/g）；

C_i—— In each standard solution, the concentration of the normal alcohol (internal standard) is a unit of micrograms per gram.（μg/g）；

R_i—— Peak area values of the normal alcohol (internal standard) in the chromatogram of each standard solution；

R_s—— Peak area values for the components to be measured in the chromatogram of each standard solution；

5——The standard solution series。

A.4.6.2 component to be measured the content of wi

The sample to be measured in the component content of WI to mg / kg (mg/kg), (A.6) is calculated according to the formula:

$$w_{\mathrm{i}} = \frac{R_u \times C_0 \times f \times 1000}{R_0 \times C_u} \quad \text{.....（A.6）}$$

Where:

Ru——The peak area of chromatogram solvent sample solution value；

C0—— In the sample solution, the concentration of the normal alcohol (internal standard), the unit is micrograms per gram；(μg/g)

C_u——The concentration of the sample solution of rosemary extract, units of micrograms per gram（μg/g）；

R0—— Peak area value of the normal alcohol (internal standard) in the chromatogram of sample solution；

f——The average response factor；

1000——Mass coefficient。

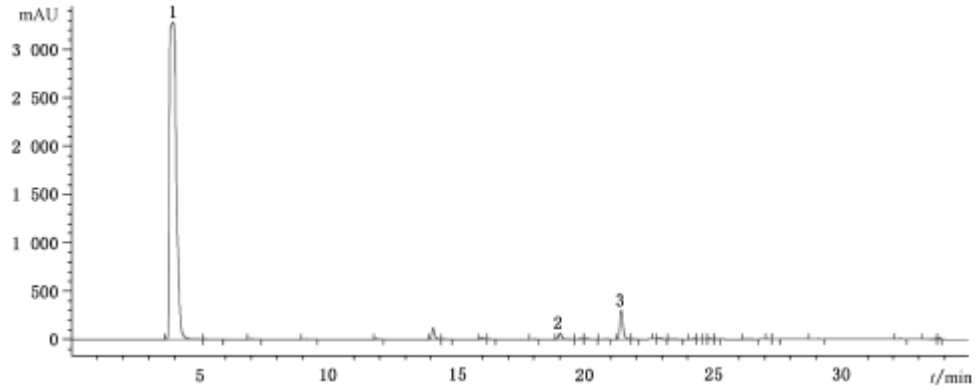
By the formula (A.6) to calculate the component to be measured (hexane and methanol) the contents of W3 and W4, both of the residual solvent content in the sample

The experimental results with the results of parallel determination of the arithmetic mean of the quasi. The absolute difference between the results obtained in two independent repeated under the condition of no more than 5 %.

Appendix B

HPLC FIG rosemary extract

Rosemary extract HPLC is shown in Figure B.1. (The Chinese are CN&CA)



1. solvent
2. carnosol
3. carnosic acid