Rosemary Extract

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CAS: [84604-14-8]
INS: 392

DESCRIPTION

Rosemary Extract may occur as a powder or a liquid. It is prepared by extraction from the leaves of *Rosmarinus officinalis*, using food grade acetone, ethanol, hexane, or a combination of hexane and ethanol (in a two-step process) as extraction solvents. It may also be extracted using supercritical carbon dioxide. Subsequent production steps may include filtration, purification, solvent evaporation, drying and sieving. Rosemary Extract may be deodorized, decolorized, and standardized using diluents and carriers permitted for use in foods. It is composed primarily of phenolic acids, flavonoids, diterpenes, diterpenoids, and triterpenes, and is characterized by the content of carnosic acid and carnosol. Rosemary Extract is insoluble in water.

Function: Flavoring agent; antioxidant

Packaging and Storage: Store in tight, light-resistant containers, protected from heat, light, moisture, and oxygen.

IDENTIFICATION

- **A. Procedure**

  Acceptance criteria: The chromatogram obtained from the *Sample solution* displays major peaks that exhibit the same relative retention times as the major peaks observed in the chromatogram of *Standard solution A*, as obtained in the *Assay* for *Carnosic Acid and Carnosol Content*.

- **B. Thin-Layer Chromatography, *Thin-Layer Chromatography, Appendix IIA***

  Sample solution: 20 mg/mL in methanol

  Standard solution A: 20 mg/mL of *USP Powdered Rosemary Extract RS* in methanol

  Standard solution B: 0.5 mg/mL of *USP Carnosic Acid RS* in methanol

  Adsorbent: 0.25-mm layer of chromatographic silica gel with a pore size of 60Å, loaded with a fluorescent indicator with an excitation wavelength of 254 nm[1]

Derivitization reagent: Carefully add 1 mL of anisaldehyde, 20 mL of glacial acetic acid, 170 mL of methanol, and 10 mL of sulfuric acid (in the exact order written) to a beaker of appropriate size. [**CAUTION—** Heat is generated; prepare with caution.]

Developing solvent system: Toluene and ethyl acetate (70:30) (v/v)

Application volume

Sample solution and Standard solution A: 1 µL

Standard solution B: 4 µL

Analysis: Using a suitable high-performance thin-layer chromatographic system, separately apply the *Sample solution*, *Standard solution A*, and *Standard solution B* to an appropriate high-performance thin-layer chromatographic plate. Develop the plate in a saturated solvent chamber (allow 15 min for chamber saturation prior to development; use plates with a controlled humidity of 33%) to a distance of 60 mm from the lower edge of the plate. Dry the plate in a stream of cool air for 10 min, then apply the *Derivitization reagent* to the plate. Heat the plate at 100° for 10 min, then examine the plate under UV light at 366 nm.

Acceptance criteria: The principal bands obtained from the *Sample solution* correspond in color, size, and R_F value to those obtained from *Standard solution A*. The *Sample solution* exhibits a band corresponding in color and R_F value to that obtained from *Standard solution B* (presence of carnosic acid).

ASSAY

● **CARNOSIC ACID AND CARNOSOL CONTENT**

Diluent: Add 0.5 mL of phosphoric acid to 100 mL of methanol.

Solution A: 0.5% phosphoric acid in water (v/v)

Mobile phase: Acetonitrile and *Solution A* (65:35)

Standard solution A: 200–500 µg/mL of *USP Powdered Rosemary Extract RS* in *Diluent*. Sonicate for 5 min, then filter through a 0.45-µm filter.

Standard solution B: 100 µg/mL of *USP Carnosic Acid RS* in *Diluent*. Sonicate for 5 min, then filter through a 0.45-µm filter.

Sample solution: 500 µg/mL in *Diluent*. Sonicate for 5 min, then filter through a 0.45-µm filter.

Chromatographic system, *Appendix IIA*

Mode: High-performance liquid chromatography

Detector: UV 230 nm

Column: 4.6-mm × 250-mm column that contains 5-µm porous silica microparticles chemically bonded to octadecylsilane[2]

Flow rate: 1.5 mL/min

Temperature: 25°

Injection size: 5 µL

System suitability

Sample: *Standard solution B*

Suitability requirement 1: The tailing factor for the carnosic acid peak is 0.90–1.30.

Suitability requirement 2: The relative standard deviation for the carnosic acid peak response (replicate injections) is NMT 2%.

Analysis: Separately inject the *Standard solutions* and the *Sample solution* into the chromatograph, and record the resulting chromatograms. Identify the peaks present in the chromatograms by comparison to the reference chromatograms supplied with the USP Reference Standards.

Calculate (separately) the percentages of carnosic acid and carnosol in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times (M_{W1}/M_{W2}) \times 100$$

r_U = peak area of the analyte of interest obtained from the chromatogram of the *Sample solution*

r_S = peak area of carnosic acid obtained from the chromatogram of *Standard solution B*

C_S = concentration of carnosic acid in *Standard solution B* (µg/mL)

C_U = concentration of the *Sample solution* (µg/mL)

F = relative response factor of the analyte (1.00 for carnosic acid; 0.92 for carnosol)

M_{W1} = molar weight of carnosic acid (332.4 g/mol)

M_{W2} = molar weight of carnosol (330.4 g/mol)

[NOTE—Add the individual percentages of carnosic acid and carnosol calculated, and report the result as the total content of carnosic acid and carnosol in the sample taken.]

Acceptance criteria: The total content of carnosic acid and carnosol is 95%–105% of the content claimed by the manufacturer.

IMPURITIES

Inorganic Impurities

- **ARSENIC, *Elemental Impurities by ICP, Method I, Appendix IIIC***

  Acceptance criteria: NMT 3 mg/kg

- **LEAD, *Elemental Impurities by ICP, Method I, Appendix IIIC***

  Acceptance criteria: NMT 2 mg/kg

Organic Impurities

- **RESIDUAL SOLVENTS**

  Solution A: 10% (w/w) solution of sodium chloride

  Internal standard solution: 160 µg/g of 1-propanol

  Standard stock solution: 260 µg/g each of ethanol and acetone

Standard solution 1: Weigh 250 mg of sunflower oil, 50 mg of the *Standard stock solution*, 2700 mg of *Solution A*, and 1000 mg of the *Internal standard solution* in a 20-mL glass autosampler vial.

Standard solution 2: Weigh 250 mg of sunflower oil, 100 mg of the *Standard stock solution*, 2650 mg of *Solution A*, and 1000 mg of the *Internal standard solution* in a 20-mL glass autosampler vial.

Standard solution 3: Weigh 250 mg of sunflower oil, 200 mg of the *Standard stock solution*, 2550 mg of *Solution A*, and 1000 mg of the *Internal standard solution* in a 20-mL glass autosampler vial.

Standard solution 4: Weigh 250 mg of sunflower oil, 500 mg of the *Standard stock solution*, 2250 mg of *Solution A*, and 1000 mg of the *Internal standard solution* in a 20-mL glass autosampler vial.

Standard solution 5: Weigh 250 mg of sunflower oil, 1000 mg of the *Standard stock solution*, 1750 mg of *Solution A*, and 1000 mg of the *Internal standard solution* in a 20-mL glass headspace vial.

Sample solution: Weigh 250 mg of Rosemary Extract, 2750 mg of *Solution A*, and 1000 mg of the *Internal standard solution* in a 20-mL glass headspace vial.

Chromatographic system, *Appendix IIA*

Mode: Headspace gas chromatography

Detector: Flame ionization

Column: 15-m × 0.15-µm column with a 0.84-µm film of 6% cyanopropylphenyl/94% dimethylpolysiloxane[3]

Carrier gas: Helium

Flow rate: 0.8 mL/min

Temperatures

Injector: 250°

Syringe headspace: 120°

Detector: 300°

Oven: Hold at 40° for 5 min; ramp to 250° at 25°/min. [NOTE— The total run time is 13.4 min.]

Headspace sampler

Sample heating temperature: 90°

Sample heating time: 10 min

Sample agitation speed: 400 rpm

Injection size: 1000 µL

Injection type: Split (1:50)

Injection speed: 1 mL/s

Injection liner: 78.5-mm × 6.3-mm (o.d.) split/splitless liner with a recessed gooseneck and a quartz wool plug; 4.0-mm (i.d.)[4]

Analysis: Place the *Sample solution* and the five *Standard solutions* into the sample tray of the headspace gas chromatograph. Record the resulting chromatograms and, using the results from the *Standard solutions*, calculate the response factors, F, for the two analytes of interest (ethanol and acetone). Separately calculate the response factors for each analyte in each of the five *Standard solutions* (five values for F will result for each of the two analytes of interest):

$$F = (C_S/C_{IS}) \times (R_{IS}/R_S)$$

C_S = concentration of the analyte of interest in the relevant *Standard solution* analyzed (µg/g)

C_{IS}= concentration of 1-propanol in the relevant *Standard solution* analyzed (µg/g)

R_{IS}= peak area response for 1-propanol obtained from the chromatogram of the relevant *Standard solution*

R_S = peak area response for the analyte of interest obtained from the chromatogram of the relevant *Standard solution*

Calculate the average response factor, F_X, for each of the two analytes:

$$F_X = \Sigma(F)/5$$

[NOTE— Add the five values F obtained above for each analyte separately, then divide each sum by the number of *Standard solutions* analyzed to generate the values for F (5) to obtain an average response factor for acetone and an average response factor for ethanol.]

Finally, separately calculate the concentrations of acetone and ethanol in the sample taken:

$$\text{Result} = F_X \times (R_U/R_{IS}) \times (C_{IS}/C_U) \times 1000$$

F_X = average response factor for the analyte of interest

R_U = peak area response for the analyte of interest obtained from the chromatogram of the *Sample solution*

R_{IS}= peak area response for 1-propanol obtained from the chromatogram of the *Sample solution*

C_{IS}= concentration of 1-propanol in the *Sample solution* (µg/g)

C_U = concentration of Rosemary Extract in the *Sample solution* (mg/g)

Acceptance criteria

Acetone: NMT 500 ppm

Ethanol: NMT 500 ppm

SPECIFIC TESTS

- **LOSS ON DRYING**

Analysis: Weigh 0.8–1.0 g of the sample into an aluminum dish in a thin layer. Load the dish into an infrared moisture analyzer, and set the instrument to run at a

temperature of 105°. At the endpoint of the analysis record the results, and calculate the percentage of the original sample weight lost in the drying process. [NOTE— Some instruments may report the result as the percentage of moisture lost. Alternatively, the *Loss on Drying* may be determined according to *Appendix IIC* at 105° until constant weight is achieved.]

Acceptance criteria: NMT 8.0%

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[1] EMD Merck HPTLC silica gel 60 F254 plates (available at http://www.emdchemicals.com), or equivalent.

[2] ZORBAX SB-C18 (Agilent Technologies), or equivalent.

[3] Agilent VF-624 MS (available from http://www.agilent.com), or equivalent.

[4] Item number 092010 from SGE Analytical Science (available at http://www.sge.com), or equivalent.

Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
ROSEMARY EXTRACT	Kristie Laurvick Senior Scientific Liaison (301) 816-8356	FI2015 Food Ingredients 2015

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