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Rhodiola crenulata Root and Rhizome

DEFINITION

Rhodiola crenulata Root and Rhizome consists of the dried roots and rhizomes of *Rhodiola crenulata* (Hook.f. & Thomson) H. Ohba (alt. name *Sedum crenulatum* Hook.f. & Thomson) (Fam. Crassulaceae), collected after the scape withers in autumn. It contains NLT 1.0% of total phenylethanoids calculated as the sum of salidroside ($C_{14}H_{20}O_7$) and tyrosol ($C_8H_{10}O_2$) on the dried basis, and NLT 0.6% of salidroside on the dried basis.

IDENTIFICATION

• A. HPTLC FOR ARTICLES OF BOTANICAL ORIGIN (203)

Standard solution: 2.0 mg/mL of [USP Salidroside RS](#) and 1.0 mg/mL of [USP Rosavin RS](#) in [methanol](#)

Sample solution: 500 mg of *Rhodiola crenulata* Root and Rhizome, finely powdered, in 5 mL of [methanol](#). Sonicate for 10 min, centrifuge, and use the supernatant.

Chromatographic system

Absorbent: Chromatographic silica gel with an average particle size of about 5 µm

Application volume: 5 µL, as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.

Developing solvent system: [Ethyl acetate](#), [methanol](#), [water](#), and [formic acid](#) (77:13:10:2)

Developing distance: 6 cm

Derivatization reagent: Dissolve 1 g of [diphenylamine](#) in 40 mL of [acetone](#), add 1 mL of aniline, and mix. Carefully add 7.5 mL of [phosphoric acid](#), and mix.

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Samples* as bands and dry in air. Develop in a saturated chamber, remove the plate from the chamber, and dry in air. Treat with *Derivatization reagent*, heat at 120° for 5 min, and examine under white light.

System suitability: The *Standard solution* shows two bands in the lower half of the chromatogram. The band with a higher R_f is due to salidroside, and the band with a lower R_f is due to rosavin.

Acceptance criteria: The *Sample solution* exhibits a band corresponding in R_f and color to salidroside in the *Standard solution*. In the *Sample solution*, no band corresponding in R_f and color to rosavin is observed (a distinction from *Rhodiola rosea*). The *Sample solution* exhibits additional bands, including two bands below salidroside but above the position corresponding to rosavin, two yellow bands in the upper half of the chromatogram, and a couple of bands close to the starting position.

• B. HPLC

Analysis: Proceed as directed in the test for *Content of Phenylethanoids*.

Acceptance criteria: The *Sample solution* exhibits a peak with a retention time corresponding to salidroside in the *Standard solution*, a peak due to tyrosol with lower intensity than salidroside, and a peak due to gallic acid. The content ratio of salidroside to tyrosol is NLT 2.

COMPOSITION

• CONTENT OF PHENYLETHANOIDS

Solution A: 0.02% [phosphoric acid](#) in [water](#)

Solution B: [Methanol](#) and [acetonitrile](#) (9:1)

Mobile phase: See [Table 1](#).

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
10	95	5
15	83	17
28	83	17
28.1	10	90
43	10	90
43.1	95	5
53	95	5

Solvent: [Methanol](#) and [water](#) (6:4)

Standard solution: 0.30 mg/mL of [USP Salidroside RS](#) in [methanol](#)

Sample solution: Accurately transfer about 500 mg of finely powdered *Rhodiola crenulata* Root and Rhizome to a suitable flask, accurately add 15.0 mL of *Solvent*, and tightly close. Weigh the filled flask with a precision of ± 0.1 mg and sonicate for 30 min. Cool to room temperature and adjust to the initial weight by adding *Solvent* if needed. Before injection, pass through a suitable membrane filter of 0.45- μ m or finer pore size and discard the first portion of the filtrate.

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: UV 275 nm

Column: 4.6-mm $\times$ 25-cm; 5- μ m packing L1

Column temperature: 25°

Flow rate: 1.0 mL/min

Injection volume: 10 μ L

System suitability

Samples: *Standard solution* and *Sample solution*

Suitability requirements

Resolution: NLT 1.5 between salidroside and tyrosol, *Sample solution*

Tailing factor: NMT 1.5 for salidroside, *Standard solution*

Relative standard deviation: NMT 2.0% for salidroside in repeated injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Using the chromatogram of the *Standard solution* and the relative retention times, identify the peaks corresponding to gallic acid, salidroside, and tyrosol in the *Sample solution*.

[NOTE—The approximate relative retention times for the peaks of gallic acid, salidroside, and tyrosol are 0.48, 1.00, and 1.05, respectively.]

Separately calculate the percentages of salidroside and tyrosol in the portion of *Rhodiola crenulata* Root and Rhizome taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times F \times 100$$

r_U = peak area of the relevant analyte from the *Sample solution*

r_S = peak area of salidroside from the *Standard solution*

C_S = concentration of [USP Salidroside RS](#) in the *Standard solution* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of *Rhodiola crenulata* Root and Rhizome taken to prepare the *Sample solution* (mg)

F = conversion factors for the analytes: 1.00 for salidroside, 0.43 for tyrosol

