

Herbal Medicines Compendium

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Citrus reticulata Peel Powder

Final Authorized Version 1.0

Citrus reticulata Peel Powder

DEFINITION

The article consists of the dried exocarp and mesocarp of the ripe fruit of *Citrus reticulata* Blanco (Family Rutaceae), partly freed from the white spongy tissue of the mesocarp, reduced to a fine or very fine powder. It contains NLT 5.0% of total dihydroflavone glycosides, calculated as the sum of narirutin (C₂₇H₃₂O₁₄), hesperidin (C₂₈H₃₄O₁₅), and didymin (C₂₈H₃₄O₁₄) on the anhydrous basis; NLT 0.1% of total polymethoxylated flavones, calculated as the sum of nobiletin (C₂₁H₂₂O₈), 3,5,6,7,8,3',4'-heptamethoxyflavone (C₂₂H₂₄O₉), and tangeretin (C₂₀H₂₀O₇) on the anhydrous basis.

POTENTIAL CONFOUNDING MATERIALS

Dried ripe fruit of *Citrus wilsonii* Tanaka

Dried unripe peel of *Citrus maxima* (Burm.) Merr. (*C. grandis* (L.) Osbeck)

CONSTITUENTS OF INTEREST

Dihydroflavone glycoside: Narirutin, hesperidin, and didymin

Polymethoxy flavone: Nobiletin, 3,5,6,7,8,3',4'-heptamethoxyflavone, and tangeretin

IDENTIFICATION

• A. BOTANICAL CHARACTERISTICS

Macroscopic: Yellowish-white powder

Microscopic: Mesocarp parenchymatous cells, numerous, irregular with unevenly thickened walls. Epidermal cells polygonal, sub-square or rectangular in surface view, 18–26 µm in diameter, anticlinal walls thickened, stomata sub-rounded, subsidiary cells indistinct; in lateral view, covered with cuticle, the outer radial wall thickened. Mesocarp parenchymatous cells containing calcium oxalate prisms in polyhedral, rhombic or biconical, 3–34 µm in diameter, 5–53 µm long; some cells containing two parallel polyhedral crystals or 3–5 prisms. Yellow or colorless hesperidin crystals in spheroid or amorphous masses and mainly present in parenchymatous cells, some of them with radial striations. Spiral, pitted, and reticulated vessels and tracheids are small.

• B. HPTLC FOR ARTICLES OF BOTANICAL ORIGIN <203>

Standard solution A: 1.0 mg/mL of USP Hesperidin RS in methanol. Sonicate to dissolve.

Standard solution B: 50 mg/mL of USP *Citrus reticulata* Pericarp Dry Extract RS in methanol. Sonicate for 20 min, centrifuge, and use the supernatant.

Sample solution: 500 mg of *Citrus reticulata* Peel Powder in 5 mL of methanol. Sonicate for 20 min, centrifuge, and use the supernatant.

Chromatographic system

Adsorbent: Chromatographic silica gel F₂₅₄ mixture

Application volume: 10 µL for *Standard solution A*; 5 µL each for *Standard solution B* and *Sample solution*, as 8-mm bands

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

Temperature: About 25°

Developing solvent system: Ethyl acetate, formic acid, and water (100:15:13)

Derivatization reagent A: 10 mg/mL of 2-aminoethyl diphenylboriate in methanol

Derivatization reagent B: 50 mg/mL of polyethylene glycol 4000 in alcohol

Analysis

Samples: *Standard solution A*, *Standard solution B*, 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 the plate at 100° for 3 min. Treat the plate with *Derivatization reagent A* and dry for 5 min with a stream of cool air. Immediately, treat the plate with *Derivatization reagent B*, dry for 5 min with a stream of cool air, and examine under UV light at 366 nm.

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements: *Standard solution A* exhibits a yellowish-brown band due to hesperidin in the lower half section. *Standard solution B* exhibits a band corresponding in R_f and color to the band due to hesperidin in *Standard solution A*, a yellow band below hesperidin, a blue band below the yellow band, another blue band above hesperidin, and a light yellowish-brown band between hesperidin and the blue band above. In the upper-third section, *Standard solution B* exhibits a bright blue band due to the coelution of nobiletin with some other components, and a faint green band above the bright blue band.

Acceptance criteria: The *Sample solution* exhibits a yellowish-brown band corresponding in R_f and color to the band due to hesperidin in *Standard solution A* and *Standard solution B*, a yellow band below hesperidin, a blue band below the yellow band, another blue band above hesperidin, and a light yellowish-brown band between hesperidin and the blue band above corresponding in R_f and color to the same bands in *Standard solution B*. In the upper-third section, the *Sample solution* exhibits a bright blue band and a faint green band above the bright blue band corresponding in R_f and color to the same bands in *Standard solution B*. In the lower-third section, the *Sample solution* exhibits a couple of yellow bands close to the starting position and a couple of faint bands above the yellow bands corresponding in R_f and color to the same bands in *Standard solution B*.

• C. HPLC

Analysis: Proceed as directed in the Assay for Content of Dihydroflavone Glycosides and Polymethoxylated Flavones.

Acceptance criteria: The *Sample solution* exhibits the most intense peak at the retention time corresponding to hesperidin in *Standard solution A* and peaks due to narirutin, didymin, nobiletin, 3,5,6,7,8,3',4'-heptamethoxyflavone, and tangeretin at retention times corresponding to the same constituents in *Standard solution B*. No other peak between narirutin and tangeretin is more intense than the peak corresponding to didymin (a distinction from other *Citrus* species; *Citrus maxima* peel and *Citrus wilsonii* fruit show a principal peak for naringin). The content ratios of narirutin and didymin relative to hesperidin are within the ranges listed in Table 2.

ASSAY

• CONTENT OF DIHYDROFLAVONE GLYCOSIDES AND POLYMETHOXYLATED FLAVONES

Solution A: 0.1% formic acid in water

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

| Time (min) | Solution A (%) | Solution B (%) |
|-------------------|-----------------------|-----------------------|
| 0 | 85 | 15 |
| 8 | 81 | 19 |
| 10 | 81 | 19 |
| 17 | 60 | 40 |
| 28 | 56 | 44 |

[NOTE—The *Standard solutions* and the *Sample solution* are stable for 24 h at room temperature.]

Standard solution A: 0.40 mg/mL of USP Hesperidin RS in methanol

Standard solution B: 0.05 mg/mL of USP Nobiletin RS in methanol

Standard solution C: 5 mg/mL of USP *Citrus reticulata* Pericarp Dry Extract RS in methanol. Sonicate for 15 min, centrifuge, and pass through a suitable membrane filter of 0.22- μ m pore size.

Sample solution: Accurately transfer about 100 mg of *Citrus reticulata* Peel Powder to a suitable flask, accurately add 10.0 mL of methanol, and close tightly. Weigh the filled flask accurately and sonicate for 30 min. Cool to room temperature and adjust to the initial weight by adding methanol if needed. Before injection, pass through a suitable membrane filter of 0.22- μ m pore size and discard the first portion of the filtrate.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 283 nm (0–17 min) and 330 nm (17–28 min)

Column: 4.6-mm \times 5-cm; 1.8- μ m packing L1 (similar to Agilent Zorbax SB C18)

Column temperature: 25°

Flow rate: 0.7 mL/min

Injection volume: 2 μ L

System suitability

Samples: *Standard solution A*, *Standard solution B*, and *Standard solution C*

Suitability requirements

Resolution: NLT 1.5 between the peaks of hesperidin and the small peak before it, *Standard solution C*

Tailing factor: NMT 1.5 for the hesperidin and nobiletin peaks, *Standard solution A* and *Standard solution B*

Relative standard deviation: NMT 2.0% for the hesperidin and nobiletin peaks, *Standard solution A* and *Standard solution B*

Chromatogram similarity: The chromatogram of *Standard solution C* is similar to the reference chromatogram provided with the lot of USP *Citrus reticulata* Pericarp Dry Extract RS being used.

Analysis

For dihydroflavone glycosides

Samples: *Standard solution A*, *Standard solution C*, and *Sample solution*

Using the chromatograms of *Standard solution A*, *Standard solution C*, and the reference chromatogram provided with the lot of USP *Citrus reticulata* Pericarp Dry Extract RS being used, identify the peaks of narirutin, hesperidin, and didymin in the *Sample solution*. [NOTE—See Table 2 for relative retention

times.]

Table 2

| Analyte | Approximate Relative Retention Time | Conversion Factor | Content Ratio Relative to Hesperidin |
|------------|-------------------------------------|-------------------|--------------------------------------|
| Narirutin | 0.8 | 1.17 | 0.1-0.3 |
| Naringin | 0.9 | — | <0.02 |
| Hesperidin | 1.0 | 1.0 | 1.0 |
| Didymin | 1.7 | 1.0 | 0.02-0.06 |

Separately calculate the percentage of narirutin, hesperidin, and didymin in the portion of *Citrus reticulata* Peel Powder 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 hesperidin from *Standard solution A*

C_S = concentration of USP Hesperidin RS in *Standard solution A* (mg/mL)

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

W = weight of *Citrus reticulata* Peel Powder taken to prepare the *Sample solution* (mg)

F = conversion factor for the analyte (see *Table 2*)

Calculate content of total dihydroflavone glycosides as the sum of the percentages of narirutin, hesperidin, and didymin.

For polymethoxylated flavones

Samples: *Standard solution B*, *Standard solution C*, and *Sample solution*

Using the chromatograms of *Standard solution B*, *Standard solution C*, and the reference chromatogram provided with the lot of USP *Citrus reticulata* Pericarp Dry Extract RS being used, identify the peaks of nobiletin, 3,5,6,7,8,3',4'-heptamethoxyflavone, and tangeretin in the *Sample solution*. [NOTE—See *Table 3* for relative retention times.]

Table 3

| Analyte | Approximate Relative Retention Time | Conversion Factor |
|-------------------------------------|-------------------------------------|-------------------|
| Nobiletin | 1.00 | 1.00 |
| 3,5,6,7,8,3',4'-Heptamethoxyflavone | 1.06 | 1.32 |
| Tangeretin | 1.12 | 0.88 |

Separately calculate the percentage of nobiletin, 3,5,6,7,8,3',4'- heptamethoxyflavone, and tangeretin in the portion of *Citrus reticulata* Peel Powder 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 nobiletin from *Standard solution B*

C_s = concentration of USP Nobiletin RS in *Standard solution B* (mg/mL)

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

W = weight of *Citrus reticulata* Peel Powder taken to prepare the *Sample solution* (mg)

F = conversion factor for the analyte (see *Table 3*)

Calculate the content of total polymethoxylated flavones as the sum of the percentages of nobiletin, 3,5,6,7,8,3',4'-heptamethoxyflavone, and tangeretin.

Acceptance criteria

Total dihydroflavone glycosides: NLT 5.0% on the anhydrous basis

Total polymethoxylated flavones: NLT 0.1% on the anhydrous basis

CONTAMINANTS

- **ARTICLES OF BOTANICAL ORIGIN <561>**, *Limits of Elemental Impurities*: Meets the requirements
- **ARTICLES OF BOTANICAL ORIGIN <561>**, *Pesticide Residue Analysis*: Meets the requirements
- **MICROBIAL ENUMERATION TESTS <61>**: The total aerobic bacterial count does not exceed 10^5 cfu/g, the total combined molds and yeasts count does not exceed 10^3 cfu/g, and the bile-tolerant Gram-negative bacteria count does not exceed 10^3 cfu/g.
- **TESTS FOR SPECIFIED MICROORGANISMS <62>**: Meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*
- **ARTICLES OF BOTANICAL ORIGIN <561>**, *Test for Aflatoxins*: Meets the requirements

SPECIFIC TESTS

- **ARTICLES OF BOTANICAL ORIGIN <561>**, *Methods of Analysis, Alcohol-Soluble Extractives, Method 1*: NLT 15.0%
- **ARTICLES OF BOTANICAL ORIGIN <561>**, *Methods of Analysis, Water-Soluble Extractives, Method 2*: NLT 30.0%
- **WATER DETERMINATION <921>**, *Method II*: NMT 12.0%
- **ARTICLES OF BOTANICAL ORIGIN <561>**, *Methods of Analysis, Total Ash*: NMT 5.0%
- **ARTICLES OF BOTANICAL ORIGIN <561>**, *Methods of Analysis, Acid-Insoluble Ash*: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light and moisture, and store at controlled room temperature.
- **LABELING:** The label states the Latin binomial following the official name of the plant contained in the article.
- **USP REFERENCE STANDARDS <11>**
 - USP *Citrus reticulata* Pericarp Dry Extract RS
 - USP Hesperidin RS
 - USP Nobiletin RS

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