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Add the following:

▲Bitter Orange Young Fruit Powder

DEFINITION

Bitter Orange Young Fruit Powder (also known as *Zhishi*) consists of the dried fallen young fruit at an early stage of development from bitter orange trees, *Citrus × aurantium* L. (Fam. Rutaceae) and its cultivars, reduced to a fine powder. It contains NLT 22% of total dihydroflavone glycosides, calculated as the sum of narinutin ($C_{27}H_{32}O_{14}$), naringin ($C_{27}H_{32}O_{14}$), hesperidin ($C_{28}H_{34}O_{15}$), and neohesperidin ($C_{28}H_{34}O_{15}$); and NLT 0.4% and NMT 1% of synephrine ($C_9H_{13}NO_2$), all on the dried basis.

IDENTIFICATION

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

Standard solution A: 0.5 mg/mL each of [USP Neohesperidin RS](#), [USP Nobiletin RS](#), and [USP Synephrine RS](#) in [methanol](#). Sonicate to dissolve.

Standard solution B: 10 mg/mL of [USP Bitter Orange Fruit Flavonoids Dry Extract RS](#) in [methanol](#). Sonicate for 15 min, centrifuge, and use the supernatant.

Sample solution: 50 mg/mL of Bitter Orange Young Fruit Powder in [methanol](#). Sonicate for 15 min, centrifuge, and use the supernatant.

Chromatographic system

(See standard parameters as defined in [HPTLC for Articles of Botanical Origin \(203\)](#), [Table 1.](#))

Application volume: 2 μ L

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

Derivatization reagent A: 3% [aluminum chloride](#) in [ethanol](#)

Derivatization reagent B: 5% [ninhydrin](#) in [ethanol](#)

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 air dry the plate.

Treat the plate with *Derivatization reagent A*, heat at 105° for 5 min, and examine under long-wave UV light. Then treat the plate with *Derivatization reagent B*, heat at 105° for 20 min, and examine under white light.

System suitability

Under long-wave UV light: *Standard solution A* exhibits a blue band due to neohesperidin in the lower-half section and a blue band due to nobiletin in the upper-third section. *Standard solution B* exhibits a blue band in the lower-third section; exhibits in the lower-half section a pair of bright blue bands, with one corresponding in R_f to neohesperidin in *Standard solution A* and another above neohesperidin due to naringin, as well as a weak blue band right below the neohesperidin band; and exhibits in the upper-third section a blue band corresponding in R_f to nobiletin in *Standard solution A* and a sky-blue band below nobiletin.

Under white light: *Standard solution A* exhibits a brown band due to synephrine in the lower-half section. *Standard solution B* exhibits a brown band corresponding in R_f to synephrine in *Standard solution A*, a brown band right above synephrine and several brown bands below synephrine.

Acceptance criteria

Under long-wave UV light: The *Sample solution* exhibits the following bands corresponding to similar bands in *Standard solution B*: a blue band in the lower-third section; in the lower-half section, a pair of bright blue bands, with one corresponding in R_f to neohesperidin in *Standard solution A* and another due to naringin above neohesperidin, as well as a weak blue band right below the neohesperidin band; and in the upper-third section, a blue band corresponding in R_f to nobiletin in *Standard solution A* (a distinction from *Citrus maxima* peel) and a sky-blue band below nobiletin (a distinction from *Citrus reticulata* peel and *C. reticulata* young fruit). The *Sample solution* also exhibits several weak bands between naringin and the sky-blue band, a faint blue band right below or partially overlapping with nobiletin, and a couple of blue bands above nobiletin.

Under white light: The *Sample solution* exhibits the following bands corresponding to similar bands in *Standard solution B*: a brown band corresponding in R_f to synephrine in *Standard solution A*, a brown band right above synephrine, and several brown bands below synephrine.

• **B. HPLC**

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

Acceptance criteria: The *Sample solution* exhibits the most intense peak corresponding to neohesperidin in *Standard solution A* (a distinction from bitter orange immature fruit, also known as *Zhiqiao*, which exhibits a most intense peak due to naringin). Also a distinction from the

peels of *C. reticulata* and *C. maxima*, which do not contain neohesperidin); peaks due to narirutin, naringin, and hesperidin corresponding to the same constituents in *Standard solution B*; and an unidentified peak with a peak intensity similar to narirutin and a relative retention time of about 0.4 compared to neohesperidin. The naringin peak is more intense than the hesperidin peak, which is more intense than the narirutin peak. Beside the above-mentioned peaks, no peak is more intense than the narirutin peak between the unidentified peak and neohesperidin peak. The respective content ratios of narirutin, naringin, and hesperidin to neohesperidin are within the ranges listed in [Table 2](#).

COMPOSITION

• CONTENT OF DIHYDROFLAVONE GLYCOSIDES

Solution A: 0.05% [phosphoric acid](#) in [water](#)

Solution B: [Acetonitrile](#)

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

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	82	18
25	82	18
26	5	95
35	5	95
36	82	18
45	82	18

Standard solution A: 0.20 mg/mL of [USP Neohesperidin RS](#) in [methanol](#). Sonicate to dissolve.

Standard solution B: 1 mg/mL of [USP Bitter Orange Fruit Flavonoids Dry Extract RS](#) in [methanol](#). Sonicate for 15 min, centrifuge, and pass through a suitable membrane filter of 0.45-µm or finer pore size.

Sample solution: Transfer about 100 mg of Bitter Orange Young Fruit Powder, accurately weighed, to a 150-mL round-bottom flask. Add 50 mL of [methanol](#), and reflux for 1 h on a water bath at 80°. Cool to room temperature, filter, and transfer the filtrate to a 100-mL volumetric flask. Wash the plant residue twice using 20 mL of [methanol](#) each time, and then filter and transfer the filtrate to the volumetric flask. Add [methanol](#) to volume and mix well. Before injection, pass through a suitable membrane filter of 0.45-µm or finer pore size, and discard the first portion of the filtrate.

Chromatographic system

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

Mode: LC

Detector: UV 284 nm

Column: 4.6-mm × 15-cm; 2.7-µm packing [L1](#)

Column temperature: 25°

Flow rate: 0.9 mL/min

Injection volume: 5 µL

System suitability

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

Suitability requirements

Resolution: NLT 3.0 between the naringin and hesperidin peaks, *Standard solution B*

Tailing factor: NMT 2.0 for the neohesperidin peak, *Standard solution A*

Relative standard deviation: NMT 2.0% for the neohesperidin peak in replicate injections, *Standard solution A*

Chromatogram similarity: The chromatogram of *Standard solution B* is similar to the reference chromatogram provided with the lot of [USP Bitter Orange Fruit Flavonoids Dry Extract RS](#) being used.

Analysis

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

Using the chromatograms of *Standard solution A* and *Standard solution B* and the reference chromatogram provided with the lot of [USP Bitter Orange Fruit Flavonoids Dry Extract RS](#) being used, identify the peaks corresponding to narirutin, naringin, hesperidin, and neohesperidin in the *Sample solution*.

Separately calculate the percentages of narirutin, naringin, hesperidin, and neohesperidin in the portion of Bitter Orange Young Fruit 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*