

Status: Currently Official on 17-Feb-2025
 Official Date: Official as of 01-Dec-2021
 Document Type: Dietary Supplement Monographs
 DocId: GUID-B17963A2-F4F9-4BB9-96FF-E61E0F091E45_3_en-US
 DOI: https://doi.org/10.31003/USPNF_M11219_03_01
 DOI Ref: n976e

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Bitter Orange Fruit Flavonoids Dry Extract

DEFINITION

Bitter Orange Fruit Flavonoids Dry Extract is prepared from the unripe fruit of *Citrus × aurantium* L. (Family Rutaceae) by extraction with hydroalcoholic mixtures. It contains NLT 45% of total citrus flavonoids on the dried basis, calculated as the sum of eriocitrin ($C_{27}H_{32}O_{15}$), neoeriocitrin ($C_{27}H_{32}O_{15}$), isonaringin ($C_{27}H_{32}O_{14}$), naringin ($C_{27}H_{32}O_{14}$), hesperidin ($C_{28}H_{34}O_{15}$), neohesperidin ($C_{28}H_{34}O_{15}$), rhoifolin ($C_{27}H_{30}O_{14}$), diosmin ($C_{28}H_{32}O_{15}$), neodiosmin ($C_{28}H_{32}O_{15}$), naringenin ($C_{15}H_{12}O_5$), didymin ($C_{28}H_{34}O_{14}$), hesperetin ($C_{16}H_{14}O_6$)-poncirin ($C_{28}H_{34}O_{14}$) (coelution), and other unspecified flavonoids. It also contains NLT 20% of naringin, NLT 10% of neohesperidin, and NMT 2.5% of hesperidin. The content ratio of naringin to neohesperidin is between 2:1 and 1:1. The content of unspecified flavonoids is NMT 4%.

IDENTIFICATION

Change to read:

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

Standard solution A: 25 mg/mL of [USP Naringin RS](#) in [methanol](#). Sonicate to dissolve.

Standard solution B: 25 mg/mL of [USP Bitter Orange Fruit Flavonoids Dry Extract RS](#) in [methanol](#). Sonicate for 20 min, centrifuge, and use the supernatant.

Sample solution: 25 mg/mL of Bitter Orange Fruit Flavonoids Dry Extract in [methanol](#). Sonicate for 20 min, centrifuge, and use the supernatant.

Chromatographic system

Absorbent: [Silica gel, precoated plates, with fluorescence indicator F₂₅₄](#) for HPTLC, size 20 cm × 10 cm

Application volume: 2 µL, 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)

Developing distance: ▲6 cm from the application line▲ (USP 1-Dec-2021)

Derivatization reagent A: 10 mg/mL of [aluminum chloride](#) in [ethyl alcohol](#)

Derivatization reagent B: 5 mg/mL of [2-aminoethyl diphenylborinate](#) in [ethyl acetate](#)

Derivatization reagent C: 50 mg/mL of [polyethylene glycol 400](#) in [methylene chloride](#)

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 110°–120° for 5 min. Treat the plate with *Derivatization reagent A* and heat at 110°–120° for 5 min. Immediately treat the warm plate with *Derivatization reagent B* and dry for 5 min with a current of cool air. Then treat with *Derivatization reagent C* and dry for 5 min in a current of cool air. After a 30-min period, examine under UV light at 366 nm and under white light.

System suitability

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

Suitability requirements: After derivatization and exposure to UV light at 366 nm, *Standard solution A* exhibits a green-blue band due to naringin in the middle-third section. *Standard solution B* exhibits a band corresponding in R_f and color to the band from naringin in *Standard solution A*, as well as a red-orange band corresponding to neoeriocitrin, which appears as a red-purple band under white light. *Standard solution B* also exhibits a faint green-blue band between naringin and neoeriocitrin (corresponding to a coelute of hesperidin and neohesperidin), a light-yellow band below neoeriocitrin, a green-blue band in the lower-third section, and a series of blue bands in the upper-third section.

Acceptance criteria: After derivatization and exposure to UV light at 366 nm, the *Sample solution* exhibits a green-blue band in the middle-third section, corresponding in R_f to the band of naringin in *Standard solution A*, as well as a red-orange band corresponding to neoeriocitrin in *Standard solution B* (differentiation from sweet orange, tangerine, and grapefruit), which appears as a red-purple band under white light. The *Sample solution* also exhibits a faint green–blue band between naringin and neoeriocitrin (corresponding to a coelution of hesperidin and neohesperidin), a light-yellow band below neoeriocitrin, a green–blue band in the lower-third section, and a series of blue bands in the upper-third section.

• **B. HPLC**

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

Acceptance criteria: The *Sample solution* exhibits two intense peaks at the retention times corresponding to naringin and neohesperidin in *Standard solution A*, while peaks due to eriocitrin, neoeriocitrin, isonaringin, hesperidin, rhoifolin, diosmin, neodiosmin, naringenin, didymin, and hesperetin–poncirin at retention times corresponding to the same constituents in *Standard solution B* are usually present. The *Sample solution* may also exhibit several unspecified flavonoid peaks, as described in [Table 1](#). Naringin and neohesperidin are the main characteristic peaks presenting the largest areas. Any other peak response is similar to or is lower than that of hesperidin. The content ratio of naringin to neohesperidin is between 2:1 and 1:1.

COMPOSITION

• CONTENT OF FLAVONOIDS

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

Mobile phase: 0.5% [glacial acetic acid](#) and [methanol](#) (70:30)

Standard solution A: 0.3 mg/mL each of [USP Naringin RS](#) and [USP Neohesperidin RS](#) in [dimethyl sulfoxide](#). Sonicate for 15 min, centrifuge, and pass through a suitable membrane filter of 0.22-µm pore size.

Standard solution B: 2 mg/mL of [USP Bitter Orange Fruit Flavonoids Dry Extract RS](#) in [dimethyl sulfoxide](#). Sonicate for 15 min, centrifuge, and pass through a suitable membrane filter of 0.22-µm pore size.

Sample solution: Accurately weigh about 40 mg of Bitter Orange Fruit Flavonoids Dry Extract and transfer to a suitable flask. Add 20.0 mL of [dimethyl sulfoxide](#), close tightly, and sonicate for 30 min. Before injection, pass through a suitable membrane filter of 0.22-µm pore size and discard the first portion of the filtrate.

Chromatographic system

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

Mode: LC

Detector: UV 284 nm

Column: 3.9-mm × 150-mm; 4-µm packing [L1](#)

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: Approximately 50 min (6.5 times the retention time of naringin)

System suitability

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

Suitability requirements

Resolution: NLT 2.0 between the naringin and hesperidin peaks; NLT 2.0 between the hesperidin and neohesperidin peaks, *Standard solution B*

Tailing factor: NMT 1.5 for naringin and neohesperidin, *Standard solution A*

Relative standard deviation: NMT 2.0% for naringin and neohesperidin in repeated 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*, *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 eriocitrin, neoeriocitrin, isonaringin, naringin, hesperidin, neohesperidin, rhoifolin, diosmin, neodiosmin, naringenin, didymin, and hesperetin–poncirin as well as peaks corresponding to unspecified flavonoids in the *Sample solution*. [NOTE—The relative retention times in [Table 1](#) are relative to naringin.]

Table 1

Analyte	Approximate Relative Retention Time	Relative Response Factor Based on Naringin	Relative Response Factor Based on Neohesperidin
Unspecified flavonoid 1	0.3	—	—
Eriocitrin	0.5	1	—
Neoeriocitrin	0.6	1	—
Isonaringin	0.8	0.91	—
Naringin	1.0	1	—
Hesperidin	1.2	1	—
Neohesperidin	1.4	—	—