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Add the following:

▲ Broccoli Seed Dry Extract

DEFINITION

Broccoli Seed Dry Extract is prepared from the seeds of *Brassica oleracea* var. *italica* Plenck (syn. *Brassica cretica* Lam.) (Fam. Brassicaceae) by aqueous extraction. It contains NLT 13% of glucoraphanin on the dried basis. It may contain suitable added substances as carriers.

IDENTIFICATION

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

Standard solution A: 1 mg/mL each of [USP Dextrose RS](#) and [USP Sucrose RS](#) in [methanol](#). Sonicate to dissolve.

Standard solution B: 1 mg/mL of [USP Glucoraphanin Potassium Salt RS](#) in [methanol](#). Sonicate to dissolve.

Standard solution C: Mix 0.1 g of [USP Broccoli Seed Dry Extract RS](#) with 10 mL of [methanol](#). Shake for 10 min at 300 rpm and centrifuge.

Sample solution: Mix 0.1 g of Broccoli Seed Dry Extract with 10 mL of [methanol](#). Shake for 10 min at 300 rpm and centrifuge.

Chromatographic system

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

Application volume: 2 µL of *Standard solution A*, 10 µL of *Standard solution B*, and 5 µL each of *Standard solution C* and *Sample solution*

Developing solvent system: [Ethyl acetate](#), [methanol](#), 0.05% aqueous [boric acid](#), and [glacial acetic acid](#) (50:30:10:2)

Derivatization reagent: Mix 4 g of [diphenylamine](#) with 160 mL of [acetone](#) and 4 mL of [aniline](#). Carefully add 30 mL of [phosphoric acid](#) to the mixture.

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, 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 warm plate with the *Derivatization reagent* for 5 min at 120° and examine under white light.

System suitability: *Standard solution A* exhibits a brown band due to sucrose and a gray band due to dextrose. *Standard solution B* exhibits a gray band due to glucoraphanin. In the lower-third section, *Standard solution C* exhibits two gray bands with increasing R_f values; the first one is due to an unknown compound and the second one is due to raffinose. In the middle-third section, *Standard solution C* exhibits a gray-blue band corresponding in R_f and color to glucoraphanin in *Standard solution B* (overlapping with glucoiberin) and a gray band corresponding in R_f and color to dextrose in *Standard solution A*. It also exhibits a broad orange band due to fructose that is observed below the dextrose band. A very faint gray band due to glucoerucin also appears in the upper-half section.

Acceptance criteria: The *Sample solution* exhibits two gray bands in the lower-third section; the first one, with a lower R_f , is due to an unknown compound, and the second one is due to raffinose. The *Sample solution* also exhibits a gray-blue band corresponding in R_f and color to glucoraphanin in *Standard solution B* (overlapping with glucoiberin). In the middle-third section, the *Sample solution* may also exhibit a brown band corresponding in R_f and color to sucrose in *Standard solution A*, a gray band corresponding in R_f and color to dextrose in *Standard solution A*, and a broad orange band between the sucrose and dextrose bands that is due to fructose. The *Sample solution* may also exhibit a faint gray band in the upper-half section due to glucoerucin.

• B. HPLC

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

Acceptance criteria: The *Sample solution* exhibits an intense peak at the retention time due to glucoraphanin in *Standard solution A*. The *Sample solution* also exhibits a series of four peaks eluting at the beginning of the chromatogram and corresponding to the same peaks in *Standard solution B*; the first peak corresponds to the solvent front, the second one is unknown, the third one is due to glucoerucin (coelute), and the fourth one is due to gluconapin. The *Sample solution* also exhibits a minor peak eluting immediately after glucoraphanin that corresponds in retention time to glucoiberin in *Standard solution B*.

COMPOSITION

• CONTENT OF GLUCORAPHANIN

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

Mobile phase: [Acetonitrile](#) and 40 mM [ammonium formate](#) (70:30), adjusted to a pH of 4.5 with formic acid

Standard solution A: 0.3 mg/mL of [USP Glucoraphanin Potassium Salt RS](#) in [acetonitrile](#) and [water](#) (70:30). Sonicate for 15 min, centrifuge, and pass through a suitable membrane filter of 0.22-µm pore size.

Standard solution B: 1 mg/mL of [USP Broccoli Seed Dry Extract RS](#) prepared as follows. Accurately weigh, in milligrams, an amount of [USP Broccoli Seed Dry Extract RS](#) equivalent to the volume, in milliliters, of the volumetric flask to be used. Transfer this portion to the flask, add

water to volume, and mix using a magnetic stir plate until fully dissolved. [NOTE—The material may take up to 30 min to dissolve.] Dilute the filtrate with *Mobile phase* (1:10). Pass an aliquot through a suitable membrane filter of 0.22-µm pore size before injection.

Sample stock solution: 1 mg/mL of Broccoli Seed Dry Extract prepared as follows. Accurately weigh, in milligrams, an amount of Broccoli Seed Dry Extract equivalent to the volume, in milliliters, of the volumetric flask to be used. Transfer this portion to the volumetric flask, add water to volume, and mix using a magnetic stir plate until fully dissolved. [NOTE—The material may take up to 30 min to dissolve.]

Sample solution: Transfer 1 mL of the *Sample stock solution* into a 10-mL flask and add *Mobile phase* to volume. Pass an aliquot through a suitable membrane filter of 0.22-µm pore size before injection.

Chromatographic system

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

Mode: LC

Detector: UV 235 nm

Columns

Guard: 2.1-mm × 20-mm; 5-µm 200 Å packing [L114](#)

Analytical: 4.6-mm × 150-mm; 5-µm 200 Å packing [L114](#)

Column temperature: 30°

Flow rate: 0.4 mL/min

Injection volume: 10 µL

Run time: 25 min

System suitability

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

Suitability requirements

Resolution: NLT 1.5 between glucoraphanin and glucoiberin, *Standard solution B*

Tailing factor: NMT 1.5 for glucoraphanin, *Standard solution A*

Relative standard deviation: NMT 2.0% for glucoraphanin 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 Broccoli Seed Dry Extract RS](#) being used.

Analysis

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

Using the chromatograms of *Standard solution A* and *Standard solution B* and the reference chromatogram provided with the lot of [USP Broccoli Seed Dry Extract RS](#) being used, identify the peak corresponding to glucoraphanin in the *Sample solution*.

Calculate the percentage of glucoraphanin in the portion of Broccoli Seed Dry Extract taken:

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

r_U = peak area of glucoraphanin from the *Sample solution*

r_S = peak area of glucoraphanin from *Standard solution A*

C_S = concentration of [USP Glucoraphanin Potassium Salt RS](#) in *Standard solution A* (mg/mL)

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

W = weight of Broccoli Seed Dry Extract taken to prepare the *Sample stock solution* (mg)

V_F = final volume of the *Sample solution* (mL)

V_A = volume of the aliquot of the *Sample stock solution* taken (mL)

Acceptance criteria: NLT 13% on the dried basis

CONTAMINANTS

- [BOTANICAL EXTRACTS \(565\)](#), [Preparations](#), [General Pharmacopeial Requirements](#), [Pesticide Residues](#): Meets the requirements
- [MICROBIAL ENUMERATION TESTS \(2021\)](#): The total aerobic bacterial count does not exceed 10^4 cfu/g, and the total combined molds and yeasts count does not exceed 10^3 cfu/g.
- [ABSENCE OF SPECIFIED MICROORGANISMS \(2022\)](#), [Test Procedures](#), [Test for Absence of Salmonella Species](#) and [Test for Absence of Escherichia coli](#): Meets the requirements

SPECIFIC TESTS

- [LOSS ON DRYING \(731\)](#)
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 8.0%
- [ARTICLES OF BOTANICAL ORIGIN \(561\)](#), [Methods of Analysis](#), [Total Ash](#): NMT 12.0%

ADDITIONAL REQUIREMENTS