Grape Seeds Oligomeric Proanthocyanidins

DEFINITION

Grape Seeds Oligomeric Proanthocyanidins is a fraction of an extract of the ripe seeds of *Vitis vinifera* L. (Fam. Vitaceae). It contains NLT 75.0% of oligomeric proanthocyanidins, on the anhydrous basis. The extract is prepared using suitable solvents such as alcohol, methanol, acetone, ethyl acetate, water, or mixtures of these solvents, in a ratio of starting plant material to extract between 70:1 and 10:1. The extract is further enriched in oligomeric proanthocyanidins by fractionation with ethyl acetate or by other means.

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST ⟨201⟩**

Adsorbent: Chromatographic silica gel with an average particle size of 5 µm and a layer thickness of about 0.2 mm (HPTLC plates)

Standard solution A: Dissolve a quantity of *USP Purified Grape Seeds Oligomeric Proanthocyanidins RS* in methanol, using sonication, to obtain a solution having a concentration of about 5 mg/mL. Centrifuge if necessary, and use the clear supernatant. [NOTE—Prepare fresh.]

Standard solution B: Dissolve a quantity of *USP (+)-Catechin RS* in methanol, using sonication, to obtain a solution having a concentration of about 1 mg/mL.

Sample solution: Proceed as directed for *Standard solution A*, except use the Grape Seeds Oligomeric Proanthocyanidins.

Developing solvent system: A mixture of acetone, toluene, and formic acid (15:15:5)

Spray reagent: Dissolve about 100 mg of vanillin in 3-mL of methanol using sonication. Add about 3 mL of hydrochloric acid, dilute with methanol to 10 mL, and carefully mix under cold water. [NOTE—Prepare fresh.]

Application volume: 15 µL, as 5–10-mm bands

Analysis

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

Apply the *Samples* as bands to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩). Use a saturated chamber. Develop the chromatograms until the solvent front has moved up about 90% of the plate. Remove the plate from the chamber, dry, spray with the *Spray reagent*, dry, and examine under visible light.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits pink-violet bands, corresponding in color and R_F to those in the chromatogram of *Standard solution A*, at the following approximate R_F values: a pair of bands between 0.20 and 0.23 (trimeric proanthocyanidins), a band at 0.28 (proanthocyanidin-B_2-3′-*O*-gallate), a band at 0.31 (B-type dimeric proanthocyanidins), and a band at 0.43 ((−)-epicatechin-3-*O*-gallate). The chromatogram of the *Sample solution* may exhibit a

pink-violet band at an approximate R_F of 0.49 (residual flavan 3-ol monomers and/or gallic acid) corresponding to the band in the chromatogram of *Standard solution B*. Other pink-violet bands may also be observed.

- **B.** The chromatogram of the *Sample solution* obtained in the test for *Limit of Catechin and Epicatechin* exhibits peaks due to proanthocyanidin dimer B_1, proanthocyanidin dimer B_2, (−)-epicatechin-3-*O*-gallate, and a broad peak due to other oligomeric proanthocyanidins at retention times corresponding to those in the chromatogram of *Standard solution B*.

COMPOSITION

- **CONTENT OF OLIGOMERIC PROANTHOCYANIDINS**

Internal standard solution: Prepare a solution of butylated hydroxytoluene in *Mobile phase* containing about 0.3 mg/mL.

Standard solution A: Dissolve a weighed quantity of *USP Purified Grape Seeds Oligomeric Proanthocyanidins RS* in *Internal standard solution* to obtain a solution having a known concentration of about 1.0 mg/mL.

Standard solution B: Dissolve a portion of *USP (+)-Catechin RS* in *Internal standard solution* to obtain a solution having a known concentration of about 0.2 mg/mL.

Sample solution: Dissolve a weighed quantity of Grape Seeds Oligomeric Proanthocyanidins in *Internal standard solution* to obtain a solution having a known concentration of about 1.0 mg/mL. Centrifuge, and use the clear supernatant.

Mobile phase: Prepare a filtered and degassed mixture of tetrahydrofuran and an aqueous solution of lithium bromide (about 1 mg/mL) (95:5).

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 7.5-mm × 30-cm; 5-µm, 500-Å, packing L21

Column temperature: 25° ± 1

Flow rate: 1.0 mL/min

Injection size: 10 µL

System suitability

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

Suitability requirements: Measure the responses as determined under *Analysis*.

Relative standard deviation: NMT 2.0% determined from the the peak area ratio of the oligomeric proanthocyanidins to the internal standard in repeated injections, *Standard solution A*

Resolution: NLT 3.0 between the peaks of monomers and the internal standard, *Standard solution B*

Analysis

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

Chromatograph *Standard solution A* and determine the beginning and end of the retention time window for the integration of oligomeric proanthocyanidins, at the points where the response of the main peak is about 0.5% of its maximum. Record the peak area ratio of the oligomeric proanthocyanidins to the internal standard. Chromatograph *Standard solution B* and the *Sample solution* and identify the locus for the monomers. Integrate the areas of the main peaks within the retention time window as determined for *Standard solution A*, excluding the area above the main peak, at the locus identified for the monomers, using a proper integration method.

Calculate the percentage of the oligomeric proanthocyanidins in the portion of the Grape Seeds Oligomeric Proanthocyanidins taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U	=	peak response ratio of the oligomeric proanthocyanidins to the internal standard from the *Sample solution*
R_S	=	peak response ratio of the oligomeric proanthocyanidins to the internal standard from *Standard solution A*
C_S	=	concentration of *USP Purified Grape Seeds Oligomeric Proanthocyanidins RS* in *Standard solution A* (mg/mL)
C_U	=	concentration of Grape Seeds Oligomeric Proanthocyanidins in the *Sample solution* (mg/mL)

Acceptance criteria: NLT 75.0% on the anhydrous basis

IMPURITIES

Inorganic Impurities

- **HEAVY METALS,** *Method II* ⟨231⟩**:** NMT 10 ppm

Organic Impurities

- **ARTICLES OF BOTANICAL ORIGIN,** *General Method for Pesticide Residues Analysis* ⟨561⟩**:** Meets the requirements

SPECIFIC TESTS

- **LIMIT OF CATECHIN AND EPICATECHIN**

Solution A: Use acetonitrile.

Solution B: Use a 0.3% aqueous solution of 85% phosphoric acid.

Solvent: Prepare a mixture of *Solution A* and *Solution B* (1:9).

Standard solution A: Dissolve, using sonication, a weighed quantity of *USP (+)-Catechin RS* in *Solvent* to obtain a solution having a known concentration of about 0.5 mg/mL.

Standard solution B: Dissolve, using sonication, a weighed quantity of *USP Grape Seeds Oligomeric Proanthocyanidins RS* in *Solvent* to obtain a solution having a known concentration of about 5 mg/mL. Centrifuge, and use the clear supernatant.

Sample solution: Proceed as directed for *Standard solution B*, except use the Grape Seeds Oligomeric Proanthocyanidins.

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	10	90
45	20	80
65	60	40
66	10	90
85	10	90

Chromatographic system

(See *Chromatography* 〈621〉*, System Suitability*.)

Mode: LC

Detector: UV 278 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 0.7 mL/min

Injection size: 10 µL

System suitability

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

Suitability requirements

The chromatogram obtained from *Standard solution B* is similar to the Reference Chromatogram provided with the lot of the *USP Grape Seeds Oligomeric Proanthocyanidins RS* being used.

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

Relative standard deviation: NMT 2% determined from the catechin peak in repeated injections, *Standard solution A*

Analysis

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

Using the chromatogram of *Standard solution A*, *Standard solution B*, and the Reference Chromatogram provided with the lot of *USP Grape Seeds Oligomeric Proanthocyanidins RS* being used, identify the retention times of the peaks corresponding to (+)-catechin and (−)-epicatechin. The approximate relative retention times of the peaks are 1.0 and 1.43 for (+)-catechin and (−)-epicatechin, respectively.

Calculate the sum of the percentages of (+)-catechin and (−)-epicatechin in the portion of the Grape Seeds Oligomeric Proanthocyanidins taken:

$$(r_U/r_S) \times (C \times V/W) \times 100$$

r_U = sum of the peak responses for (+)-catechin and (−)-epicatechin from the *Sample solution*

r_S = peak response for (+)-catechin in *Standard solution A*

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

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

W = weight of Grape Seeds Oligomeric Proanthocyanidins taken to prepare the *Sample solution* (mg)

Acceptance criteria: NMT 19.0% on the anhydrous basis

- **MICROBIAL ENUMERATION TESTS** ⟨2021⟩**:** The total aerobic microbial count does not exceed 10^4 cfu/g. The total combined yeast and mold count does not exceed 10^3 cfu/g.
- **ABSENCE OF SPECIFIED MICROORGANISMS** ⟨2022⟩**:** It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.
- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.5%, determined on 5.0 g
- **WATER,** *Method Ia* ⟨921⟩**:** NMT 8.0%
- **WATER-INSOLUBLE FRACTION**

  Analysis: Transfer about 1 g, weighed, to a suitable flask. Add 100 mL of water, and shake vigorously for 15 min. Pass the solution through a previously tared sintered-glass filter, wash the flask with 30 mL of water, and transfer the washings to the filter. Wash the filter with 30 mL of water in 5-mL portions. Dry the filter for 2 h at 105°, cool in a desiccator, and weigh. Calculate the percentage of the water-insoluble fraction.

  Acceptance criteria: NMT 2%
- **OTHER REQUIREMENTS:** It meets the requirements of the test for *Residual Solvents* under *Botanical Extracts* ⟨565⟩.

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 and, following the official name, it states "Grape Seeds Oligomeric Proanthocyanidins". It meets other labeling requirements under *Botanical Extracts* ⟨565⟩.
- **USP REFERENCE STANDARDS** ⟨11⟩

  USP (+)-CATECHIN RS

  USP GRAPE SEEDS OLIGOMERIC PROANTHOCYANIDINS RS

  USP PURIFIED GRAPE SEEDS OLIGOMERIC PROANTHOCYANIDINS RS

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Maged H. Sharaf, Ph.D. Principal Scientific Liaison 1-301-816-8318	(DS2010) Monographs - Dietary Supplements
《2021》	Radhakrishna S Tirumalai, Ph.D. Principal Scientific Liaison 1-301-816-8339	(GCM2010) General Chapters - Microbiology
《2022》	Radhakrishna S Tirumalai, Ph.D. Principal Scientific Liaison 1-301-816-8339	(GCM2010) General Chapters - Microbiology
Reference Standards	RS Technical Services 1-301-816-8129 rstech@usp.org	

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Chromatographic Column—

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Chromatographic columns text is not derived from, and not part of, USP 34 or NF 29.