Status: Currently Official on 16-Feb-2025
Official Date: Official as of 01-May-2018
Document Type: NF Monographs
DocId: GUID-E20BE1A6-75C1-4F5A-A845-AF85332C9D3B_3_en-US
DOI: https://doi.org/10.31003/USPNF_M58620_03_01
DOI Ref: v2n37

Olive Oil

CAS RN®: 8001-25-0.

DEFINITION

Olive Oil is the refined fixed oil obtained from the ripe fruit of *Olea europaea* L. (Fam. Oleaceae). It may contain suitable antioxidants.

IDENTIFICATION

• **A. Identity by Fatty Acid Composition**

Analysis: Proceed as directed in the test for *Fats and Fixed Oils ⟨401⟩, Fatty Acid Composition*.

Acceptance criteria: Meets the composition profile of fatty acids in *Table 1*

• **B. Identity by Triglyceride Profile**

Analysis: Proceed as directed in the test for *Identification of Fixed Oils by Thin-Layer Chromatography ⟨202⟩*.

Acceptance criteria: Meets the requirements in the chapter

IMPURITIES

• **Alkaline Impurities**

Sample: 10 mL of Olive Oil

Analysis: Mix 10 mL of freshly opened acetone and 0.3 mL of water, and add 0.05 mL of bromophenol blue TS. Add the *Sample*, shake, and allow to stand. Titrate with 0.01 N hydrochloric acid VS to change the color of the upper layer to yellow.

Acceptance criteria: NMT 0.1 mL of 0.01 N hydrochloric acid is required.

SPECIFIC TESTS

• **Fats and Fixed Oils,** *Acid Value (Free Fatty Acids) ⟨401⟩*: NMT 0.3. [Note—Petroleum ether with a 100°–120° boiling range can be used to replace ether in the test.]

• **Fats and Fixed Oils,** *Peroxide Value ⟨401⟩*: NMT 10.0

• **Fats and Fixed Oils,** *Fatty Acid Composition ⟨401⟩*: Olive Oil exhibits the composition profile of fatty acids shown in *Table 1*, as determined in the chapter.

Table 1

Carbon-Chain Length	Number of Double Bonds	Percentage (%)
<16	0	≤0.1
16	0	7.5–20.0
16	1	≤3.5
18	0	0.5–5.0
18	1	56.0–85.0
18	2	3.5–20.0
18	3	≤1.2

Carbon-Chain Length	Number of Double Bonds	Percentage (%)
20	0	≤0.7
20	1	≤0.4
22	0	≤0.2
24	0	≤0.2

- **Absence of Sesame Oil**

  Sample: 10 mL of Olive Oil

  Analysis: Mix the *Sample* with a mixture of 0.5 mL of a 0.35% (v/v) solution of furfural in acetic anhydride and 4.5 mL of acetic anhydride, and shake the mixture for about 1 min. Pass through a filter paper previously wetted with acetic anhydride. Add 0.2 mL of sulfuric acid to the filtrate.

  Acceptance criteria: No bluish-green color develops.

- **Fats and Fixed Oils, *Unsaponifiable Matter* ⟨401⟩:** NMT 1.5%. [Note—Petroleum ether with a 40°–60° boiling range can be used to replace ether in the test.]

- **Ultraviolet Absorbance**

  Sample solution: Dissolve 1.0 g of Olive Oil in cyclohexane, and dilute with cyclohexane to 100 mL.

  Instrumental conditions

  (See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

  Mode: UV-Vis

  Wavelength: 270 nm

  Path length of the cell: 1 cm

  Analysis: Determine the UV-Vis absorbance using the *Instrumental conditions* described above.

  Acceptance criteria: The absorbance is NMT 1.20.

- **Water Determination, *Method Ic* ⟨921⟩:** NMT 0.1%

- **Sterol Composition**

  2 M Alcoholic potassium hydroxide solution: Dissolve 12 g of potassium hydroxide in 10 mL of water, and dilute with alcohol (ethanol) to 100 mL.

  Sample A: Accurately weigh 5 g of Olive Oil into a 150-mL flask fitted with a reflux condenser. Add 50 mL of *2 M Alcoholic potassium hydroxide solution*, and heat on a water bath for 1 h, shaking frequently. Add 50 mL of water through the top of the condenser, shake, and allow to cool. Transfer the contents of the flask to a separating funnel. Rinse the flask with several portions totaling 50 mL of petroleum ether with a 40°–60° boiling range, and add the rinsings to the separating funnel. Shake vigorously for 1 min. Allow to separate, and transfer the aqueous layer to a second separating funnel. If an emulsion forms, add small quantities of alcohol or a concentrated solution of potassium hydroxide. Shake the aqueous layer with two 50-mL quantities of petroleum ether with a 40°–60° boiling range. Combine the petroleum ether layers in a third separating funnel and wash with three 50-mL quantities of 50% alcohol. Transfer the petroleum ether layer to a tared 250-mL flask. Rinse the separating funnel with small quantities of petroleum ether with a 40°–60° boiling range, and add to the flask. Evaporate the petroleum ether on a water bath and dry the residue at 100°–105° for 15 min, keeping the flask horizontal. Allow to cool in a desiccator and weigh.

  Reference A: Accurately weigh 5 g of sunflower oil into a 150-mL flask fitted with a reflux condenser. Proceed as directed for *Sample A*, beginning with "Add 50 mL of *2 M Alcoholic potassium hydroxide solution*".

  Separation of the sterol fraction by LC

  Mobile phase: Isopropyl alcohol and *n*-hexane (1:99)

  Sample solution A: Transfer *Sample A* with three 4-mL quantities of petroleum ether with a 40°–60° boiling range to a 15-mL test tube. [Note—Ether can be used to replace petroleum ether if *Sample A* is not well soluble in petroleum ether.] Evaporate to dryness under a stream of nitrogen. Dissolve *Sample A* in *Mobile phase* to obtain a solution with an approximate concentration of 40 mg/mL. Add a few drops of isopropyl alcohol to improve the solubility. [Note—3 drops are normally sufficient to ensure complete solubilization.] Pass through a membrane filter (nominal 0.45-µm pore size).

  Reference solution A: Prepare as directed for *Sample solution A*, except use *Reference A* instead of *Sample A*.

  Chromatographic system

  (See *Chromatography* ⟨621⟩ .)

  Mode: LC

Detector: UV 210 nm

Columns

Guard: 4.6-mm × 0.5-cm (or 4.6-mm × 1.0-cm); 5-µm packing L3, with a 6-nm pore size

Analytical: 4.6-mm × 25-cm; 5-µm packing L3, with a 6-nm pore size

Flow rate: 1.0 mL/min

Injection volume: 50 µL

Identification of the peaks due to sterols

Samples: *Sample solution A* and *Reference solution A*

Sterol identification: The sterol fraction elutes at the end of the chromatogram. Locate the fraction to be collected by using the chromatogram from *Reference solution A*. The chromatogram from *Reference solution A* shows two or three principal peaks, which elute at approximately 21–35 min depending on the column used. The chromatogram from *Sample solution A* may have one principal peak.

Sterol collection: Collect the fraction at the detector outlet in a 15-mL tube with a screw cap. Evaporate the solvent under a stream of nitrogen. [NOTE—If necessary, to increase the sample amount for later analysis, make the second injection of 50 µL on the HPLC column and collect the fraction at the detector outlet in the same 15-mL test tube with a screw cap. Evaporate the solvent under a stream of nitrogen.]

Determination of sterols by GC

Sample solution B: Dissolve the residue of the sterol fraction obtained from *Sample solution A* in the previous LC step in 0.2 mL of anhydrous pyridine and 0.2 mL of a mixture of 1 volume of chlorotrimethylsilane and 99 volumes of bis(trimethylsilyl)trifluoroacetamide. Insert the stopper into the test tube tightly, and heat at 80° for 20 min. Allow to cool and use the liquid phase.

Reference solution B: Dissolve 9 parts of the residue of the sterol fraction obtained from *Reference solution A* in the previous LC step and 1 part of cholesterol in 0.2 mL of anhydrous pyridine and 0.2 mL of a mixture of 1 volume of chlorotrimethylsilane and 99 volumes of bis(trimethylsilyl)trifluoroacetamide. Insert the stopper into the test tube tightly and heat at 80° for 20 min. Allow to cool and use the liquid phase.

Chromatographic system

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

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 30-m fused-silica capillary; 0.25-µm layer of phase G27

Temperatures

Injection port: 290°

Detector: 290°

Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
260	—	260	38
260	5	290	5

Carrier gas: Helium

Flow rate: 2.6 mL/min

Injection volume: 1–3 µL (depending on the expected amount of sterols in the test sample)

Injection type: Split injection; split ratio is 25:1

System suitability

Sample: *Reference solution B*

The chromatogram from *Reference solution B* shows five principal peaks corresponding to cholesterol, campesterol, stigmasterol, β-sitosterol, and Δ7-stigmastenol.

[NOTE—The retention times of the sterols with reference to β-sitosterol are given in *Table 3*.]

Table 3

Identification	Relative Retention Time
Cholesterol	0.65
Brassicasterol	0.71
24-Methylene-cholesterol	0.80
Campesterol	0.82
Campestanol	0.83
Stigmasterol	0.87
Δ7-Campesterol	0.92
Δ5,23-Stigmastadienol	0.95
Clerosterol	0.96
β-Sitosterol	1.00
Sitostanol	1.01
Δ5-Avenasterol	1.03
Δ5,24-Stigmastadienol	1.09
Δ7-Stigmastenol	1.13
Δ7-Avenasterol	1.17

Suitability requirements

Resolution: NLT 3.0 between the campesterol and stigmasterol peaks

Analysis

Samples: *Sample solution B* and *Reference solution B*

Use the chromatogram from *Reference solution B* to identify the peaks due to cholesterol, campesterol, stigmasterol, β-sitosterol, and Δ7-stigmastenol. Identify the peaks due to the sterols in the chromatogram from *Sample solution B* using the chromatograms from *Reference solution B* and the relative retention times with reference to β-sitosterol (main peak) given in *Table 3*.

Calculate the percentage content of each sterol in the sterol fraction of Olive Oil taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = area of the peak due to the sterol component to be determined

r_T = sum of the areas of the peaks due to the components indicated in *Table 3*

Acceptance criteria: Olive Oil exhibits the composition profiles of sterols shown in *Table 4*.

Table 4

Component	Percentage (%)
Cholesterol	≤0.5

Component	Percentage (%)
Campesterol	≤4.0
Δ7-Stigmastenol	≤0.5
Sum of the contents of Δ5,23-stigmastadienol, clerosterol, β-sitosterol, sitostanol, Δ5-avenasterol, and Δ5,24-stigmastadienol	≥93.0

The content of stigmasterol is NMT that of campesterol.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant, well-filled containers, and prevent exposure to excessive heat.
- **Labeling:** Label it to indicate the name and quantity of any suitable antioxidants.

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

Topic/Question	Contact	Expert Committee
OLIVE OIL	Documentary Standards Support	CE2020 Complex Excipients
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	CE2020 Complex Excipients

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. PF 40(4)

Current DocID: GUID-E20BE1A6-75C1-4F5A-A845-AF85332C9D3B_3_en-US
Previous DocID: GUID-E20BE1A6-75C1-4F5A-A845-AF85332C9D3B_1_en-US
DOI: https://doi.org/10.31003/USPNF_M58620_03_01
DOI ref: v2n37