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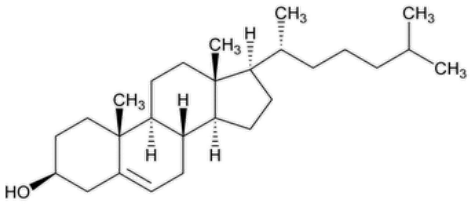
Cholesterol



$C_{27}H_{46}O$ 386.65

Cholest-5-en-3-ol, (3β)-;

Cholest-5-en-3β-ol CAS RN®: 57-88-5.

DEFINITION

Cholesterol is a steroid alcohol containing NLT 95.0% and NMT 102.0% of cholest-5-en-3β-ol ($C_{27}H_{46}O$), calculated on the dried basis. It may contain suitable antioxidants.

IDENTIFICATION

- **A.** **Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K**
- **B.** It meets the requirements of the test for *Optical Rotation ⟨781S⟩, Procedures, Specific Rotation*.
- **C.** It meets the requirements of the test for *Melting Range or Temperature ⟨741⟩*.

ASSAY

- **Procedure**

Standard solution: 1.0 mg/mL of USP Cholesterol RS and 1.0 mg/mL of pregnenolone isobutyrate (internal standard) in heptane

Sample solution: 1.0 mg/mL of Cholesterol and 1.0 mg/mL of pregnenolone isobutyrate (internal standard) in heptane

Chromatographic system

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

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 30-m capillary bonded with a 0.25-µm layer of phase G2

Temperatures

Injection port: 285°

Column: 275°

Detector: 300°

Carrier gas: Helium

Flow rate: 2.0 mL/min

Injection volume: 1.0 µL

Injection type: Split ratio, 25:1

Liner: Cup splitter liner (4 mm × 6.3 × 78.5) with deactivated glass wool

System suitability

Sample: *Standard solution*

[Note—The relative retention times for pregnenolone isobutyrate and cholesterol are 1.0 and 1.2, respectively.]

Suitability requirements

Resolution: NLT 10 between pregnenolone isobutyrate and cholesterol

Relative standard deviation: NMT 2.0% for the peak response ratio of cholesterol to the internal standard

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of cholesterol in the portion of sample taken:

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

R_U = peak response ratio of cholesterol to the internal standard (peak response of cholesterol/peak response of the internal standard) from the *Sample solution*

R_S = peak response ratio of cholesterol to the internal standard (peak response of cholesterol/peak response of the internal standard) from the *Standard solution*

C_S = concentration of USP Cholesterol RS in the *Standard solution* (mg/mL)

C_U = concentration of Cholesterol in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–102.0% on the dried basis

IMPURITIES

• **Residue on Ignition ⟨281⟩:** NMT 0.1%

• **Limit of Related Sterols and Other Organic Impurities**

Internal standard solution: 0.02 mg/mL of pregnenolone isobutyrate (internal standard) in heptane

System suitability solution: 0.02 mg/mL of USP Cholesterol RS, 0.04 mg/mL of desmosterol, and 0.02 mg/mL of lathosterol in *Internal standard solution*

Sample solution: 2.0 mg/mL of Cholesterol in *Internal standard solution*

Chromatographic system: Proceed as directed in the *Assay*.

System suitability

Sample: *System suitability solution*

[Note—See *Table 1* for the relative retention times.]

Table 1

Name	Relative Retention Time
Pregnenolone isobutyrate (internal standard)	1.00
Cholesterol	1.23
Desmosterol (cholesta-5,24-dien-3β-ol)	1.31
Lathosterol (5α-cholest-7-en-3β-ol)	1.34

Suitability requirements

Resolution: NLT 2.0 between desmosterol and lathosterol

Relative standard deviation: NMT 5.0% for peak response ratio of desmosterol to the internal standard

Analysis

Samples: *System suitability solution* and *Sample solution*

Three more related sterols may be observed (see *Table 2*).

Table 2

Name	Relative Retention Time
Pregnenolone isobutyrate (internal standard)	1.00
β-Cholestanol (5α-cholestan-3β-ol, dihydrocholesterol)	1.24

Name	Relative Retention Time
24-Dehydrolathosterol (5α-cholesta-7,24-dien-3β-ol)	1.42
4-Methylcholest-5-en-3β-ol	1.51

Calculate the percentage of desmosterol or lathosterol in the portion of Cholesterol taken:

$$\text{Result} = (R_{U1}/R_{S1}) \times (C_{S1}/C_U) \times 100$$

R_{U1} = peak response ratio of desmosterol or lathosterol to the internal standard (peak response of desmosterol or lathosterol/peak response of the internal standard) from the *Sample solution*

R_{S1} = peak response ratio of desmosterol or lathosterol to the internal standard (peak response of desmosterol or lathosterol/peak response of the internal standard) from the *System suitability solution*

C_{S1} = concentration of desmosterol or lathosterol in the *System suitability solution* (mg/mL)

C_U = concentration of Cholesterol in the *Sample solution* (mg/mL)

Calculate the percentage of β-cholestanol, 24-dehydrolathosterol, 4-methylcholest-5-en-3β-ol, or any other unspecified organic impurity in the portion of Cholesterol taken:

$$\text{Result} = (R_{U2}/R_{S2}) \times (C_{S2}/C_U) \times 100$$

R_{U2} = peak response ratio of β-cholestanol, 24-dehydrolathosterol, 4-methylcholest-5-en-3β-ol, or any other unspecified impurity to the internal standard (peak response of β-cholestanol, 24-dehydrolathosterol, 4-methylcholest-5-en-3β-ol, or any other unspecified impurity/peak response of the internal standard) from the *Sample solution*

R_{S2} = peak response ratio of cholesterol to the internal standard (peak response of cholesterol/peak response of the internal standard) from the *System suitability solution*

C_{S2} = concentration of USP Cholesterol RS in the *System suitability solution* (mg/mL)

C_U = concentration of Cholesterol in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 3*. Disregard peaks less than 0.05% for any unspecified impurities and any peaks due to solvent.

Table 3

Name	Acceptance Criteria (%)
β-Cholestanol (5α-cholestan-3β-ol, dihydrocholesterol)	≤0.6
Desmosterol	≤4
Lathosterol	≤2
24-Dehydrolathosterol (5α-cholesta-7,24-dien-3β-ol)	≤0.2
4-Methylcholest-5-en-3β-ol	≤0.5
Total impurities including related sterols	≤5

SPECIFIC TESTS

- **Melting Range or Temperature ⟨741⟩:** 147°–150°
- **Optical Rotation ⟨781S⟩,** *Procedures, Specific Rotation*

Sample solution: 20 mg/mL, undried, in dioxane

Acceptance criteria: −34° to −38°

Change to read:

• **Acidity**

Sample: 1.0 g

Analysis: Dissolve the *Sample* in 10 mL of ether in a small flask, add 10.0 mL of 0.10 N sodium hydroxide, and shake for about 1 min. Heat gently to expel the ether, then boil for 5 min. Cool, dilute with 10 mL of water, add phenolphthalein TS, and titrate with 0.10 N sulfuric acid until the pink color just disappears, stirring the solution vigorously throughout the titration. Perform a blank determination (see ▲*Titrimetry ⟨541⟩*▲ (CN 1-Aug-2024)).

Acceptance criteria: The difference between the number of mL of 0.10 N sulfuric acid consumed in the blank and the number of mL consumed in the *Sample* is NMT 0.3 mL.

• **Loss on Drying ⟨731⟩**

Analysis: Dry under vacuum at 60° for 4 h.

Acceptance criteria: NMT 0.3%

• **Solubility in Alcohol**

Sample: 500 mg

Analysis: Dissolve the *Sample* in 50 mL of warm alcohol in a stoppered flask or cylinder, and allow to stand at room temperature for 2 h.

Acceptance criteria: No deposit or turbidity is formed.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed, light-resistant containers.

• **Labeling:** Label it to indicate whether cholesterol is derived from animal, synthetic, or vegetable sources. For animal-derived sources, indicate the species and tissue used (for example, bovine brain and spinal cord, wool fat, or chicken eggs). Indicate the names and amounts of any added antioxidants.

• **USP Reference Standards ⟨11⟩**

USP Cholesterol RS

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

Topic/Question	Contact	Expert Committee
CHOLESTEROL	Documentary Standards Support	CE2020 Complex Excipients

Chromatographic Database Information: Chromatographic Database

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