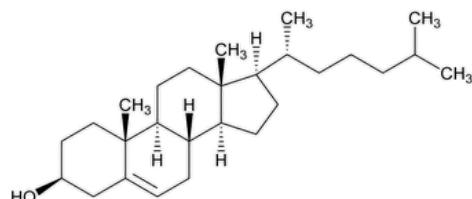


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Cholesterol



$C_{27}H_{46}O$ 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-3 β -ol)	1.31
Lathosterol (5 α -cholest-7-en-3 β -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 (5 α -cholestan-3 β -ol, dihydrocholesterol)	1.24