National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material 911b

Cholesterol

This Standard Reference Material (SRM) is certified as a chemical of known purity. It is intended primarily for use in the calibration and standardization of procedures for the determination of cholesterol in clinical samples and for routine evaluations of daily working standards used in these procedures. The SRM is supplied in a unit containing 2 g.

Cholesterol certified value, purity 99.8 ± 0.1%

The purity and estimated uncertainty is based upon scientific judgment and evaluation of the numerous analytical tests applied to this SRM in the certification process. The uncertainty given approximates two standard deviations about the certified value.

Source of Material: The cholesterol used for this SRM was prepared by and obtained from JBL Scientific, Inc., San Luis Obispo, CA.

Analyses were performed at NIST by G.D. Byrd, S.N. Chesler, R.G. Christensen, A. Cohen, B. Coxon, M.J. Welch, and E. White V of the NIST Organic Analytical Research Division and D.A. Becker and G. Marinenko of the NIST Inorganic Analytical Research Division.

The overall direction and coordination of the technical measurements leading to the certification were provided by A. Cohen of the NIST Organic Analytical Research Division.

Statistical consultation was provided by R.C. Paule, NIST National Measurement Laboratory.

The technical and support aspects concerning the preparation, certification, and issuance of this SRM were coordinated through the Standard Reference Materials Program by R.L. McKenzie. Revision of this certificate was coordinated through the Standard Reference Materials Program by J.C. Colbert.

Sample Preparation and Homogeneity: Companion steroids were removed by treatment with bromine, dehalogenation with zinc and recrystallization from methanol [1]. Homogeneity of this SRM was determined by gas chromatography-mass spectrometry and differential scanning calorimetry. Purity was also determined by these techniques and by a number of additional analyses performed to characterize the material.

Information Values: Additional analyses were performed to characterize the material. Results of these measurements are described below and are provided for informational purposes only. These values are not certified but are provided because they may be of interest to the user of the SRM.

Gaithersburg, MD 20899
April 15, 1994
(Revision of certificate dated 5-12-88)

Thomas E. Gills, Chief
Standard Reference Materials Program

(over)

Samples of the SRM were derivatized with N,O-bis(trimethylsilyl)acetamide and examined by gas chromatography-mass spectrometry using a 30 m, non-polar capillary column. Three impurities were detected which had retention times and apparent molecular weights identical to authentic 7-dehydrocholesterol (0.03%), campesterol (0.05%) and sitosterol (0.05%). Two common impurities in cholesterol, lathosterol and cholestanol, were not observed at an estimated detection level of 0.02%.

No impurities were detected by infrared absorption or nuclear magnetic resonance. The ultraviolet absorption spectrum (4% solution in dichloromethane) showed faint traces of peaks with approximate λ_{max} at 296, 309, 324, and 350 nm.

Differential scanning calorimetry indicated 0.095 mole percent impurity. Thin-layer chromatography on silica gel GF using 9:1 (v/v) chloroform-acetone, spraying with 20% aqueous phosphoric acid, and heating at 110 °C gave three trace spots, invisible, but fluorescent under 365-nm UV light. The R_f (Retardation factors) values of the spots relative to cholesterol were 1.86, 1.22, and 0.56. The melting point in a sealed tube under vacuum was 149.0-149.2 °C. The specific rotation was -39.94°(c, 4.0; $CHCl_3$); where c is the concentration of cholesterol in $CHCl_3$ at 4 g per 100 mL $CHCl_3$. Insoluble matter, determined by filtering a chloroform solution using a porcelain crucible of 1.5 µm absolute retention, was 0.017%. Weight loss on vacuum drying at 60 °C and 0.05 Torr (6.7 Pa) was 0.02%. Total ash content by combustion was 0.006% (850 °C). Neutron activation analysis indicated a bromine content of approximately 38 µg/g, and a chlorine content of 6 µg/g. Iodine was less than 0.1 µg/g.

WARNING: This SRM is for "in vitro" diagnostic use only.

STABILITY AND STORAGE OF THIS SRM: The SRM should be stored in a tightly-closed bottle kept in a freezer (-6 °C to 24 °C). It should not be subjected to heat, direct sunlight or sources of ultraviolet radiation. For extended periods of storage after opening, the material should be kept at or below -15 °C in a desiccator under inert gas. It should be allowed to warm to room temperature before opening. If this procedure is followed, drying is unnecessary. Experience at NIST, where SRM 911a was stored under inert gas at -15 °C, indicated that SRM 911b stored under the same conditions may be stable for as many as 10 years. If the purity of the material degrades beyond the limits certified, purchasers will be notified by NIST. If the material is stored in a refrigerator (2-8 °C), it is recommended that the material should not be used after 3 years from the date of shipment from NIST. If it is stored in the dark at room temperture, it is recommended that the material not be used after six months from the date of shipment from NIST.

PREPARATION OF STOCK STANDARD SOLUTION: A stock standard solution of cholesterol in ethanol (5 mmol/L) may be prepared by dissolving 193.7 mg of SRM 911b in 50 mL of warm absolute ethanol in a 100 mL volumetric flask, allowing the solution to cool, and diluting to exactly 100 mL with ethanol [2]. The 5 mmol/L solution of cholesterol in ethanol should be stored in an all-glass, tightly-stoppered bottle at 0 °C. Under such conditions this solution should be stable for about 4 months [3].

Solutions of cholesterol in glacial acetic acid gradually form cholesteryl acetate when stored and errors may result when using this solution [4].

All constituted solutions of cholesterol should be clear and display no turbidity.

REFERENCES

[1] Fieser, L.F., Cholesterol, Δ^5-Cholesten-3-one, and Δ^4-Cholesten-3-one, Org. Synth; 1955, 35, 43-49.

[2] Fundamental of Clinical Chemistry, N. Tietz, (ed.), W.B. Saunders Co., Philadelphia, 1970, p. 358.

[3] Henry, R.D., Clinical Chemistry, Principles and Technics, Hoeber Medical Division, Harper & Rowe, New York, 1967, p. 854.

[4] Klein, B. and Kleinman, N.B., Esterification of Cholesterol in Glacial Acetic Acid, Clin. Chem., 1974, 20, 90-91.