U. S. Department of Commerce
Philip M. Klutznick
Secretary

National Bureau of Standards
Ernest Ambler, Director

National Bureau of Standards

Certificate of Analysis

Standard Reference Material 911a

Cholesterol

A. Cohen, W. May, and R. Schaffer

This Standard Reference Material is certified as a chemical of known purity. It is intended primarily for use in the calibration and standardization of procedures for cholesterol determinations employed in clinical analysis and for routine critical evaluation of the daily working standards used in these procedures.

Purity . . . . . 99.8 ± 0.1 percent

The selenium dioxide test for lathosterol [1] was negative.

The purity was determined by the use of liquid chromatography, gravimetric recovery, and thin-layer chromatography. The most recent monitoring of purity, completed in February 1980, was performed by the use of mass spectrometry, capillary column gas chromatography, and thin-layer chromatography. The results showed nonsignificant changes in the levels of impurities.

This Standard Reference Material should be stored in the tightly capped bottle in a desiccator at 0 °C. It should be allowed to warm to room temperature before opening. If this procedure is followed, drying is unnecessary.

The cholesterol used for this Standard Reference Material was obtained from the J. T. Baker Chemical Company of Phillipsburg, N.J. Analyses and physical determinations were performed by A. Cohen, S. P. Cram, E. R. Deardorff, D. H. Freeman, H. S. Hertz, W. May, R. Schaffer, W. P. Schmidt, C. P. Talley, and J. K. Taylor. The additional analyses were performed by S. Chesler, B. Coxon, L. Sniegoski, and E. White V.

The overall direction and coordination of technical measurements leading to the certification were under the chairmanship of R. Schaffer.

The technical and support aspects concerning the preparation, certification, and issuance of this Standard Reference Material were coordinated through the Office of Standard Reference Materials by T. W. Mears.

Washington, D.C. 20234
March 5, 1980
(Revision of Certificate
dated 11-1-74)

George A. Uriano, Chief
Office of Standard Reference Materials

(over)

Liquid chromatography was performed on this material using a column packed with poly-2-methyl-5-vinylpyridine/divinylbenzene. The sample size was exactly 1 g cholesterol (measured to the nearest 0.1 mg). The eluant was chloroform and the progress of the elution was monitored by the measurement of transmittance of effluent at 254 nm. Two peaks due to impurities were detected at 0.7 and 0.8 retention time relative to the cholesterol peak. Recovery of the sample by gravimetry corrected for the blank due to column bleed, was 100.0_4 percent. The weighed effluent residues were examined by thin-layer chromatography on 250-μm layers of silica gel using 1:1 (v/v) ethyl acetate--heptane as developing solvents. Spraying with 1:5 (v/v) sulfuric acid--methanol followed by heating to 110 °C revealed two impurities at migration distances of 1.53 and 1.16 relative to that of cholesterol. These impurities, found in fractions representing a combined weight of 0.08 percent of the intial cholesterol, corresponded to the impurities detected by prior liquid chromatography at retention times of 0.7 and 0.8, respectively.

Gas-chromatographic examination, using Gas-Chrom Q treated with 3 percent OV-17 as the stationary phase at 240 °C, showed impurity peaks at retention times 0.5, 0.80, and 1.2 relative to cholesterol.

Non-destructive neutron activation analysis of cholesterol samples sealed in high-purity quartz ampoules prior to irradiation indicated a bromine content of 12 μg/g, a chlorine content of 0.7 μg/g, and an iodine content of less than 0.8 μg/g. The estimated accuracy of these measurements was within 20 percent.

The infrared-absorption spectrum of SRM 911a was the same as that of SRM 911. The ultraviolet-absorption spectrum of SRM 911a (4 percent solution in methylene chloride) showed very little absorption in the range 400 to 240 nm. This absorption is less than that exhibited by SRM 911 under similar conditions.

The melting point of this material, measured in a sealed tube under vacuum, is 149.0 to 149.4 °C. The specific rotation $[a]_D^{20}$ is −0.700 rad (−40.1°) (c, 1; $CHCl_3$). Microchemical analysis found carbon 83.86 ± 0.05 wt. percent, hydrogen 12.00 ± 0.05 weight percent; theoretical percentages based on $C_{27}H_{46}O$ are 83.87 and 11.99, respectively.

Heating the material at 100 °C at 3Pa (0.02 mm Hg) resulted in a nearly constant loss in weight of 0.01 percent per hour over a 1.5 day period. Such a volatilization may be related to degradation and/or sublimation processes.

Precautions

This Standard Reference Material is intended for "in vitro" diagnostic use only.

The Standard Reference Material should be stored in a tightly closed bottle kept in a refrigerator or freezer. It should not be subjected to heat, direct sunlight, or artificial sources of ultraviolet radiation. For extended periods of storage after opening, it is recommended that the material be kept at or below −15 °C in a desiccator under inert gas. Experience at NBS, where the material is stored under inert gas at −15 °C, indicates the material may be stable for 10 years. If the purity of control material at NBS degrades beyond the limits certified, purchasers will be notified by NBS. However, if the material is stored under ordinary laboratory conditions in a refrigerator, it is recommended that the material should not be used after 3 years from the date of purchase. If it is stored in the dark at room temperature, it is recommended that the material not be used after six months from the date of purchase.