U. S. Department of Commerce
Malcolm Baldrige
Secretary

National Bureau of Standards
Ernest Ambler, Director

National Bureau of Standards

Certificate of Analysis

Standard Reference Material 1595

Tripalmitin

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 chemical analysis of serum for triglycerides, and for the critical evaluation of routine working or secondary reference materials used in these procedures. The certified tripalmitin content is given below with associated uncertainty that is based on the expected upper limit for bias between the high performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR) methods used in the certification.

The constituents in parentheses are not certified, and the uncertainties reported are plus or minus one standard deviation of the mean.

Tripalmitin	99.5	±0.2	weight percent
Unknown glyceride	(0.5	±0.1)	weight percent
Methanol	(0.0057	±0.0002)	weight percent
Insoluble matter	(0.0020	±0.0009)	weight percent
Residue on ignition	(0.001	±0.0005)	weight percent

NOTICE AND WARNINGS TO USERS

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

Storage: SRM 1595 should be stored in the tightly capped bottle at 25° C or less. It should be allowed to warm to room temperature before opening. Under proper storage, this material should be stable for at least five years. If the purity of the material degrades beyond the limits certified, purchaser will be notified by NBS. This material is not certified for use after five years from date of purchase.

The tripalmitin used for this SRM was obtained from Nu-Chek-Prep, Inc., Elysian, Minnesota.

Analyses and physical determinations were performed at NBS in the Organic Analytical Research Division by A. Cohen, B. Coxon, M.L. Luzarraga, S. Margolis, L.T. Sniegoski, and E. White V.

Microchemical analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tennessee, and Schwarzkopf Microanalytical Laboratory, Woodside, New York.

The statistical analysis of the data was made by R. Paule, NBS National Measurement Laboratory.

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

The technical and support aspects involved in the preparation, certification, and issuance of this Standard Reference Material were coordinated through the Office of Standard Reference Materials by R. Alvarez.

Washington, D.C. 20234
July 6, 1983

Stanley D. Rasberry, Chief
Office of Standard Reference Materials

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The identity of the SRM was confirmed by proton and ^{13}C NMR spectroscopy, by the observation of a molecular ion at a mass to charge ratio of 806 in its electron impact mass spectrum, and by melting point 68.2-69.0° C (uncorrected).

The tripalmitin content of the SRM was determined by HPLC to be 99.5% and by proton NMR spectroscopy to be 100.0%. In each case, the tripalmitin value was calculated by subtraction of the impurities determined by the respective methods from 100%. For example, the tripalmitin content determined by proton NMR was obtained by subtraction of the methanol content (0.0057%). (The contents of insoluble matter and residue on ignition make negligible contributions to the calculation of tripalmitin content.) Apart from methanol, no other impurities were detected by direct NMR spectroscopy of the SRM, and therefore, the proton integral remaining after subtraction of the methanol signal from the total integral measured by NMR was used as a measure of the tripalmitin content of the SRM.

The HPLC method resolves positional isomers of mixed triglycerides, O-acetyl-di-O-palmitylglycerols, and di-O-acetyl-O-palmitylglycerols, and was used to assess both the purity and homogeneity of the SRM. For this purpose, ten selected samples of the SRM were analyzed by HPLC and five of these samples were selected randomly for duplicate determinations.

HPLC of the SRM showed a strong peak for tripalmitin with retention constant (capacity factor) $k' = 11.25$ and a weak impurity peak at $k' = 6.33$. Retention constant $k' = \frac{\text{Elution Volume - Void Volume.}}{\text{Void Volume}}$ The intensity of the impurity peak was below the detection threshold of the HPLC integrator in use, and so, for the purpose of purity and homogeneity testing, the HPLC data was acquired and processed by means of an NMR data acquisition system. The proportion of impurity was calculated as the ratio of the peak areas of the impurities to the sum of the peak areas of the impurities plus the tripalmitin. The assumption was made that tripalmitin and the impurity have a similar absorbance at 215 nm. This assumption is reasonable for saturated triglycerides of similar structure.

The purity of the SRM was additionally assessed by thin layer chromatography (TLC). Under certain specific conditions, the SRM showed an intense spot for tripalmitin at R_f 0.30 and a very faint impurity spot at R_f 0.09. The mobility of the impurity did not correspond to that of palmitic acid, palmityl alcohol, methyl palmitate, 1-O-palmitylglycerol, 2-O-palmitylglycerol, 1,2-di-O-palmitylglycerol, 1,3-di-O-palmitylglycerol, tri-O-acetylglycerol, 2,3-di-O-acetyl-1-O-palmitylglycerol, 1,3-di-O-acetyl-2-O-palmitylglycerol, 3-O-acetyl-1,2-di-O-palmitylglycerol, or 2-O-acetyl-1,3-di-O-palmitylglycerol.

Proton NMR spectroscopy of an impurity fraction isolated by repeated HPLC of the SRM indicated that the impurity is most likely a triglyceride of similar structure. No signals for olefinic protons were detected in the spectrum of the impurity concentrate thus ruling out the possibility of an unsaturated triglyceride.

The integrations of the HPLC peaks for tripalmitin and the unknown glyceride indicated satisfactory homogeneity for the SRM.

The content of insoluble matter in the SRM was determined by dissolution and filtration of three, three-unit pools of the SRM (~6 g each) in chloroform (80 mL) that had been prefiltered through a micropore filter (type FH, 0.5 μm).

The residue on ignition was determined by volatilization of three, three-unit pools of the SRM (~6 g each) from covered, tared 30-mL platinum crucibles followed by two treatments of the residues with 100 μL of concentrated sulfuric acid and ignition of the crucibles at 800 ± 25° C for 15 min.

Microchemical analysis yielded these percentages: carbon, 75.96 ± 0.42; hydrogen, 12.25 ± 0.05. Calculated percentages based on $C_{51}H_{98}O_6$ are 75.87 and 12.24, respectively, and the reported uncertainties are plus or minus one standard deviation of the mean.

A stock solution of tripalmitin may be prepared by a method similar to that used for triolein (1). Dissolve 0.100 g of tripalmitin in chloroform contained in a 100-mL-volumetric flask and dilute to volume with chloroform. Tightly stoppered, this stock standard solution is stable for several months in the dark.

A working tripalmitin standard solution should be prepared daily before use by diluting one volume of stock solution with nine volumes of chloroform. A standard quantity of glycerol may be generated from the working solution by saponification according to an available procedure (1).

This Standard Reference Material has been measured and certified at the laboratories of the National Bureau of Standards, Gaithersburg, Maryland. All inquiries should be addresssed to:

Office of Standard Reference Materials
Room B311 Chemistry Building
National Bureau of Standards
Washington, DC 20234

The date of issuance and certification of this Standard Reference Material was June, 1983.

References

1. Wybenga, D.R. and Inkpen, J.A., Clinical Chemistry Principles and Technics, second edition, R.J. Henry, D.C. Cannon, and J.W. Winkelman, Harper & Row, Hagerstown, Md., 1974, p. 1458.