National Institute of Standards & Technology

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. A unit of SRM 1595 consists of 2 g of material.

Certified Value: The certified tripalmitin content is given below with the 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.

	Weight Percent (%)		
Tripalmitin	99.5	±	0.2

Reference Values: The following weight percent values are not certified and the uncertainty provided are plus or minus one standard deviation of the mean.

	Weight Percent (%)		
Unknown glyceride	0.5	±	0.1
Methanol	0.0057	±	0.0002
Insoluble matter	0.0020	±	0.0009
Residue on ignition	0.001	±	0.0005

Expiration of SRM Certificate: The certification of **SRM 1595** is valid, within the measurement uncertainty specified, for five years from date of purchase, provided the SRM is handled and stored in accordance with instructions given in this certificate (see "Instructions for Storage and Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Coordination of the technical measurements leading to the certification of this SRM was performed by B. Coxon formerly of the NBS Organics Analytical Research Division.

This Certificate of Analysis has undergone editorial revision to reflect program and organizational changes at NIST and at the Department of Commerce. No attempt was made to reevaluate the certificate values or any technical data presented on this certificate.

Carlos Gonzalez, Chief
Chemical Sciences Division

Gaithersburg, MD 20899
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Certificate Revision History on Last Page

Steven J. Choquette, Acting Director
Office of Reference Materials

Analytical measurements were performed at NBS in the former Organics Analytical Research Division by A. Cohen, B. Coxon, M. Luzarraga, S. Margolis, L.T. Sniegoski, and E. White V. Microchemical analysis were performed by Gaibraith Laboratories, Inc. (Knoxville, TN) and Swarzkopf Microanalytical Laboratory (Woodside, NY).

Statistical analysis of the data was provided by R. Paule of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

SOURCE, PREPARATION AND ANALYSIS[1]

The tripalmitin was obtained from Nu-Chek-Prep, Inc. (Elysian, MN).

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 °C to 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 spectroscopy 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' = (Elution Volume - Void Volume)/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 additional 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-palmitriglycerol, 2-O-palmitriglycerol, 1,2-di-O-palmitriglycerol, 1,3-di-O-palmitriglycerol, 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 integrators 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 FY, 0.5 µm).

[1] Certain commercial equipment, instruments or materials are identified in this certificate to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

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 °C ± 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.

NOTICE AND WARNING TO USERS

SRM 1595 IS INTENDED FOR RESEARCH USE.

INSTRUCTIONS FOR STORAGE AND USE

Storage: SRM 1595 should be stored in a tightly-closed bottle at or below room temperature (−20 °C to 23 °C is recommended). For extended periods of storage after opening, the material should be kept at or below room temperature in a desiccator under inert gas. It should be allowed to warm to room temperature before opening.

Use: A stock solution of tripalmitin may be prepared by a method similar to that used triolein [1]. Dissolve 0.100 g of tripalmitin in chloroform contained in a 100-mLvolumetric 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 dilution 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].

REFERENCES

[1] Wybenga, D.R.; Inkpen, J.A.; *Clinical Chemistry Principles and Technics*; 2nd ed.; Henry, R.J; Cannon, D.C.; Winkelman, J.W., Eds.; Harper & Row: Hagerstown, MD, p. 1458 (1974).

Certificate Revision History: 12 May 2016 (Updated storage conditions; editorial changes); 06 January 2016 (Editorial changes); 06 July 1983 (Original certificate date).

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail mailto:srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.