National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 3278

Tocopherols in Edible Oils

This Standard Reference Material (SRM) is intended primarily for use in validating analytical methods for the determination of tocopherols in edible oils and similar matrices. This SRM can also be used for quality assurance when assigning values to in-house control materials. A unit of SRM 3278 consists of five ampoules of oil each containing approximately 1 mL of material under argon.

The development of SRM 3278 was a collaboration between the National Institute of Standards and Technology (NIST) and the National Institutes of Health (NIH), Office of Dietary Supplements (ODS).

Certified Mass Fraction Values: The certified mass fraction values of tocopherols are provided in Table 1. Certified values were derived from the combination of results provided by NIST using three or four analytical methods. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1]. The certified values in this material are the equally weighted means of the individual sets of NIST results; the associated uncertainties are expanded uncertainties at the 95 % level of confidence [2,3]. Certified values are reported on an "as-received" basis in mass fraction units [4].

Expiration of Certification: The certification of **SRM 3278** is valid, within the measurement uncertainty specified, until **30 August 2027**, 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 before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate this notification.

Coordination of the technical measurements leading to the certification of this SRM was performed by L.C. Sander, and S.A. Wise of the NIST Chemical Sciences Division and K.E. Sharpless of the NIST Special Programs Office.

Acquisition and preparation of the material were coordinated by K.E. Sharpless.

Analytical measurements at NIST were performed by C.A. Rimmer of the NIST Analytical Chemistry Division and K. Putzbach, formerly of NIST.

Support for the development of SRM 3278 was provided in part by the NIH Office of Dietary Supplements. Technical consultation was provided by J.M. Betz (NIH-ODS).

Statistical analysis was provided by J.H. Yen of the NIST Statistical Engineering Division.

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

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NOTICE AND WARNING TO USERS

SRM 3278 IS INTENDED FOR RESEARCH USE ONLY, NOT FOR HUMAN CONSUMPTION.

INSTRUCTIONS FOR STORAGE AND USE

Storage: Unopened ampoules of SRM 3278 should be stored at controlled room temperature (20 °C to 25 °C), in the dark, until required for use.

Use: Prior to removal of a test portion for analysis, the contents of the ampoule should be mixed thoroughly. The recommended minimum sample size is 0.05 g; test portions used for NIST analyses described below were 0.05 g to 2 g.

SOURCE, PREPARATION, AND ANALYSIS[1]

Material Acquisition and Preparation: Vegetable oils were combined to give an α-tocopherol/γ-tocopherol peak-area ratio of approximately 1:1. This entailed blending 10 % soybean oil, 10 % canola oil, 10 % safflower oil, and 70 % sunflower oil (mass fractions). Butylated hydroxytoluene (BHT) was added as an antioxidant at a final mass fraction of approximately 500 mg/kg.

Analytical Approach for Determination of Tocopherols: Value assignment of the mass fractions of tocopherols in SRM 3278 was based on the combination of measurements from three different analytical methods at NIST using monomeric C_{30}, polymeric C_{30}, and naphthalene LC columns with absorbance and/or fluorescence detection. Four independently prepared calibrants were used for each of the methods. Calibrant solutions containing the individual tocopherols were prepared gravimetrically at levels intended to approximate the levels of the tocopherols in the SRM. A single internal standard solution was used for the calibrants and samples. Samples were diluted in ethanol containing an internal standard (tocol) prior to analysis; a few drops of isopropanol were added to increase the oil's solubility.

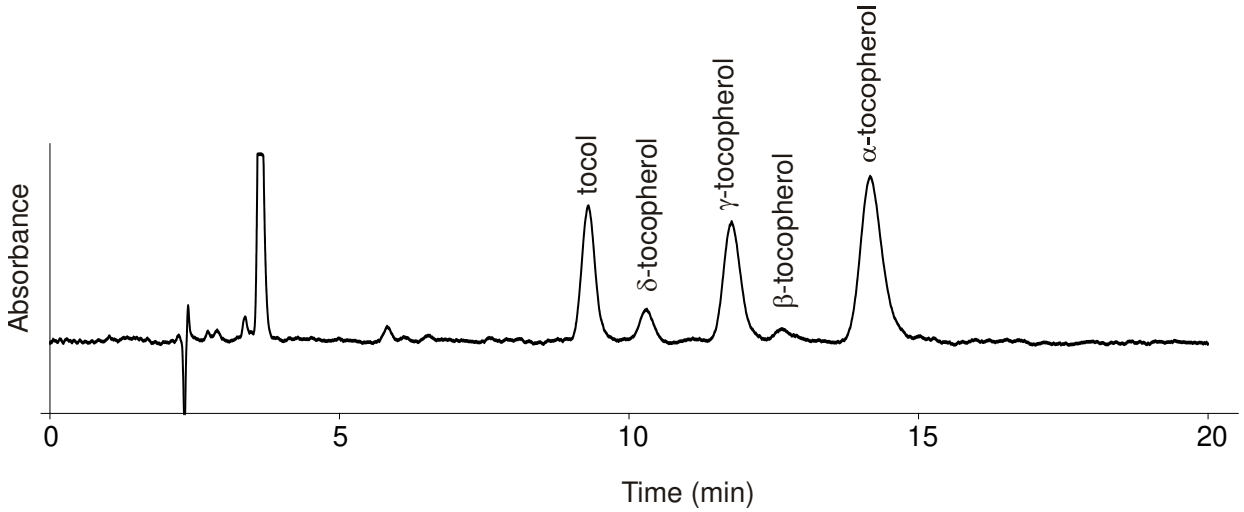

Figure 1. A sample chromatogram showing the separation on the naphthalene column (Cosmosil π-NAP, 250 mm × 4.6 mm, 5 μm particle size Nacalai Tesque Co., Japan) using absorbance detection at 297 nm and an isocratic mobile phase of 90 % methanol and 10 % water (volume fractions) is provided in the figure. The column was held at 5 ºC with a recirculating water bath.

Homogeneity Assessment: The homogeneity of tocopherols was assessed at NIST by using the monomeric C_{30} column with fluorescence detection. An analysis of variance did not show inhomogeneity for 0.05 g to 2 g test portions.

Value Assignment: The equally weighted means from each set of data were used to calculate the assigned values. Because of interferences, three methods were used to provide values for α-, β-, and γ-tocopherol; all methods were used for δ-tocopherol. Analytical methods are identified in Table 1.

[1] Certain commercial instruments, materials, or processes 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 instruments, materials, or processes identified are necessarily the best available for the purpose.

Certified Mass Fraction Values: Each certified mass fraction value is an equally weighted mean of the individual sets of results provided by NIST. The uncertainty in the certified value, calculated according to the method described in the ISO/JCGM Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-method and within-method components of uncertainty. The coverage factor, $k = 2$, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and approximately 95 % confidence for each analyte. The measurand is the certified mass fraction value reported on an "as-received" basis in mass fraction units [4]; metrological traceability is to the derived SI unit for mass fraction expressed as micrograms per gram.

Table 1. Certified Mass Fraction (as-received) Values for Tocopherols in SRM 3278

Tocopherol	Mass Fraction (μg/g)
α-Tocopherol[a,b,c]	290.1 ± 6.5
β-Tocopherol[a,b,d]	11.38 ± 0.52
γ-Tocopherol[b,c,d]	111.5 ± 5.8
δ-Tocopherol[a,b,c,d]	28.8 ± 1.8

[a] Monomeric C_{30} column with fluorescence detection (excitation at 298 nm and emission at 325 nm)
[b] Polymeric C_{30} column with fluorescence detection (excitation at 298 nm and emission at 325 nm)
[c] Naphthalene column with absorbance detection (297 nm)
[d] Naphthalene column with fluorescence detection (excitation at 298 nm and emission at 325 nm)

REFERENCES

[1] May, W.; Parris, R.; Beck, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260–136, U.S. Government Printing Office: Washington, DC (2000); available at http://www.nist.gov/srm/upload/SP260-136.PDF (accessed Aug 2016).

[2] JCGM 100:2008; *Evaluation of Measurement Data - Guide to the Expression of Uncertainty in Measurement;* (GUM 1995 with Minor Corrections), Joint Committee for Guides in Metrology (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Aug 2016); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297; U.S. Government Printing Office: Washington, DC (1994); available at http://www.nist.gov/pml/pubs/index.cfm (accessed Aug 2016).

[3] Levenson, M.S.; Banks, D.L.; Eberhardt, K.R.; Gill, L.M.; Guthrie, W.F.; Liu, H.-K.; Vangel, M.G.; Yen, J.H.; Zhang, N.F.; *An Approach to Combining Results From Multiple Methods Motivated by the ISO GUM;* J. Res. Natl. Inst. Stand. Technol., Vol. 105, pp. 571–579 (2000).

[4] Thompson, A.; Taylor, B.N.; *Guide for the Use of the International System of Units (SI)*; NIST Special Publication 811; U.S. Government Printing Office: Washington, DC (2008); available at http://www.nist.gov/pml/pubs/sp811/index.cfm (accessed Aug 2016).

Certificate Revision History: 09 August 2016 (Change expiration date; editorial changes); 03 January 2014 (Change expiration date; editorial changes); 10 December 2009 (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 srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.