

Standard Reference Material[®] 3275

Omega-3 and Omega-6 Fatty Acids in Fish Oil

CERTIFICATE OF ANALYSIS

Purpose: This Standard Reference Material (SRM) is intended primarily for validation of methods for determining fatty acids in fish oils and similar materials. This SRM can also be used for quality assurance when assigning values to in-house reference materials.

Description: SRM 3275 consists of three individual oils: Part 3275-1, a concentrate high in docosahexaenoic acid (DHA); Part 3275-2, an anchovy oil high in DHA and eicosapentaenoic acid (EPA); and Part 3275-3, a concentrate containing 60 % long-chain omega-3 fatty acids. A unit of SRM 3275 consists of two ampoules of each of the three oils, each ampoule containing approximately 1.2 mL of material.

Certified Values: The certified mass fraction values for fatty acids are provided in Table 1. 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]. Analyses for value assignment were performed by NIST using two independent methods. All certified values are calculated as the mean of the mean values from each method. The associated uncertainties are expressed at the 95 % level of confidence [2–4]. Values are reported on an as-received basis in mass fraction units [5].

Non-Certified Values: Non-certified values are provided in Appendix A.

Additional Information: Additional information is provided in Appendix B.

Period of Validity: The certified values delivered by **SRM 3275** are valid within the measurement uncertainty specified until **31 December 2028**. The certified values are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its validity. If substantive technical changes occur that affect the certification, NIST will issue an amended certificate through the NIST SRM website (<https://www.nist.gov/srm>) and notify registered users. SRM users can register online from a link available on the NIST SRM website or fill out the user registration form that is supplied with the SRM. Registration will facilitate notification. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (<https://www.nist.gov/srm>).

Table 1. Certified Mass Fraction Values for Fatty Acids as FAMES^(a,b)

	Part 3275-1 Mass Fraction (mg/g)	Part 3275-2 Mass Fraction (mg/g)	Part 3275-3 Mass Fraction (mg/g)
Dodecanoic Acid (C12:0; Lauric Acid)			0.95 ± 0.12
Tetradecanoic Acid (C14:0; Myristic Acid)	1.094 ± 0.053	3.45 ± 0.40	67.9 ± 1.5
Hexadecanoic Acid (C16:0; Palmitic Acid)	5.25 ± 0.35	8.01 ± 0.44	186.9 ± 9.4
(Z)-9-Hexadecenoic Acid (C16:1 n-7) (Palmitoleic Acid)	7.43 ± 0.24	5.83 ± 0.45	85.7 ± 3.1
Octadecanoic Acid (C18:0; Stearic Acid)	4.22 ± 0.13	12.94 ± 0.62	38.0 ± 5.7
(Z)-9-Octadecenoic Acid (C18:1 n-9) (Oleic Acid)	11.25 ± 0.93	22.1 ± 1.6	112.3 ± 2.6
(Z)-11-Octadecenoic Acid (C18:1 n-7) (Vaccenic Acid)	5.33 ± 0.35	9.24 ± 0.77	38.5 ± 2.2
ω-6 (Z,Z)-9,12-Octadecadienoic Acid (C18:2 n-6) (Linoleic Acid)	2.31 ± 0.19	3.00 ± 0.42	13.49 ± 0.45
Eicosanoic Acid (C20:0; Arachidic Acid)		0.357 ± 0.027	1.14 ± 0.26
(Z)-11-Eicosenoic Acid (C20:1 n-9) (Gondoic Acid)		6.66 ± 0.69	2.92 ± 0.14
ω-3 (Z,Z,Z,Z)-5,8,11,14,17-Eicosapentaenoic Acid (C20:5 n-3; EPA)	113 ± 12		
Docosanoic Acid (C22:0; Behenic Acid)	4.02 ± 0.24	1.396 ± 0.046	0.502 ± 0.047
(Z)-13-Docosenoic Acid (C22:1 n-9; Erucic Acid)		3.43 ± 0.32	

^(a) Each certified mass fraction value (as received) is the mean from the combination of the mean from each set of results from analyses by NIST using GC/MS and GC-FID. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. It expresses both the observed difference between the results from the methods and their respective uncertainties, consistent with the ISO/JCGM Guide and its supplement 1 [2–4]. The expanded uncertainty is calculated as $U = k u_c$ where u_c is intended to represent, at the level of one standard deviation, the effects of the combined components of uncertainty, and where the coverage factor $k = 2$ for a 95 % confidence interval (approximately) for each analyte.

^(b) The measurand is the mass fraction for the fatty acids listed as FAMES. The certified values are metrologically traceable to the International System of Units (SI) derived unit for mass fraction, expressed as milligrams per gram.

Safety: FOR RESEARCH USE; NOT FOR HUMAN CONSUMPTION.

Storage: The SRM should be stored under refrigeration (2 °C to 8 °C), in an unopened ampoule, until required for use. The certification does not apply to contents of previously opened and stored ampoules, as the stability of fatty acids has not been investigated.

Use: Before use, the contents of the ampoule should be mixed thoroughly. For certified values to be valid, test portions of at least 0.5 g should be used.

REFERENCES

- [1] Beauchamp, C.R.; Camara, J.E.; Carney, J.; Choquette, S.J.; Cole, K.D.; DeRose, P.C.; Duewer, D.L.; Epstein, M.S.; Kline, M.C.; Lippa, K.A.; Lucon, E.; Molloy, J.; Nelson, M.A.; Phinney, K.W.; Polakoski, M.; Possolo, A.; Sander, L.C.; Schiel, J.E.; Sharpless, K.E.; Toman, B.; Winchester, M.R.; Windover, D.; *Metrological Tools for the Reference Materials and Reference Instruments of the NIST Material Measurement Laboratory*; NIST Special Publication 260-136, 2021 edition; National Institute of Standards and Technology, Gaithersburg, MD (2021); available at <https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2021.pdf> (accessed Jan 2024).
- [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 (JCGM) (2008); available at <https://www.bipm.org/en/committees/jc/jcgm/publications> (accessed Jan 2024); 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 <https://www.nist.gov/pml/nist-technical-note-1297> (accessed Jan 2024).
- [3] JCGM 101:2008; *Evaluation of measurement data – Supplement 1 to the “Guide to the expression of uncertainty in measurement” - Propagation of distributions using a Monte Carlo method*; JCGM (2008); available at <https://www.bipm.org/en/committees/jc/jcgm/publications> (accessed Jan 2024).
- [4] Efron, B.; Tibshirani, R.J.; *An Introduction to the Bootstrap*; Chapman & Hall, London, UK (1993).
- [5] 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 <https://www.nist.gov/pml/special-publication-811> (accessed Jan 2024).

Certificate Revision History: **16 January 2024** (Change of period of validity; updated format; editorial changes); **29 November 2018** (Change of expiration date; editorial changes); **08 June 2016** (Updated reference to 3275-1, 3275-2, and 3275-3 as Parts instead of SRMs; editorial changes); **07 January 2015** (Change of expiration date; editorial changes); **28 December 2012** (Certified values changed to reference values for EPA and ALA in Part 3275-2 and Part 3275-3; certified values changed to reference values for DPA and DHA; certified value changed to reference value for Lignoceric Acid in Part 3275-2; reference values updated for some unsaturated fatty acids; editorial changes); **13 September 2010** (Original certificate issue date).

Certain commercial equipment, instruments, or materials may be identified in this Certificate of Analysis 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.

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, MD 20899-2300; telephone (301) 975-2200; e-mail srminfo@nist.gov; or the Internet at <https://www.nist.gov/srm>.

***** End of Certificate of Analysis *****

APPENDIX A

Non-Certified Values: Non-certified fraction values for additional fatty acids are provided in Table A1. A NIST non-certified value is the best estimate of the true value based on available data; however, the value does not meet the NIST criteria for certification [1] and is provided with associated uncertainties that may reflect only measurement reproducibility, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods. Values are reported on an as-received basis in mass fraction units [5].

Table A1. Non-Certified Mass Fraction Values for Fatty Acids as FAMES^(a,b)

	Part 3275-1			Part 3275-2			Part 3275-3			
	Mass Fraction		<i>k</i>	Mass Fraction		<i>k</i>	Mass Fraction		<i>k</i>	
	(mg/g)			(mg/g)			(mg/g)			
	(Z)-9-Tetradecenoic Acid (C14:1 n-5; Myristoleic Acid)						0.964 ± 0.043	2.09		
ω-3	(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3 n-3) (α-Linolenic Acid; ALA)		1.21 ± 0.05	2.57	1.42 ± 0.12		2.57	6.61 ± 0.31	2.57	
	Eicosanoic Acid (C20:0; Arachidic Acid)			1.910 ± 0.071 ^(c)			2.26			
ω-6	(Z,Z,Z,Z)-5,8,11,14-Eicosatetraenoic Acid (C20:4 n-6) (Arachidonic Acid)		5.69 ± 0.19	2.57	22.9 ± 1.0		2.57			
	(Z)-13-Docosenoic Acid (C22:1 n-9; Erucic Acid)			4.76 ± 0.22			2.09			
ω-6	(Z,Z,Z)-6,9,12-Octadecatrienoic Acid (C18:3 n-6) (γ-Linolenic Acid; GLA)		0.344 ± 0.025	2.57	0.507 ± 0.043		2.57	1.771 ± 0.099	2.11	
	Tetracosanoic Acid (C24:0; Lignoceric Acid)						0.618 ± 0.028	2.57	0.441 ± 0.013	2.57
	(Z)-15-Tetracosenoic Acid (C24:1 n-9; Nervonic Acid)						3.78 ± 0.29			2.57
ω-3	(Z,Z,Z,Z,Z)-5,8,11,14,17-Eicosapentaenoic Acid (C20:5 n-3; EPA)					394 ± 17	2.57	154 ± 9	2.57	
	(Z,Z,Z,Z,Z)-7,10,13,16,19-Docosapentaenoic Acid (C22:5; DPA)		70.2 ± 1.1	2.57	67.6 ± 2.3		2.57	27.0 ± 1.1	2.57	
ω-3	(Z,Z,Z,Z,Z,Z)-4,7,10,13,16,19-Docosahexaenoic Acid (C22:6 n-3; DHA)		429 ± 15	2.57	187 ± 8		2.57	104 ± 5	2.57	

^(a) Each non-certified mass fraction value (as received) is the mean of a single set of results from analyses by NIST using GC-FID, except where noted. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence, consistent with the ISO/JCGM Guide [2]. The uncertainty incorporates within-measurement uncertainty. The expanded uncertainty is calculated as $U = ku_c$ where u_c is intended to represent, at the level of one standard deviation, the effects of the combined components of uncertainty, and k is a coverage factor corresponding to approximately 95 % confidence for each analyte.

^(b) The measurand is the mass fraction for the fatty acids listed as FAMES.

^(c) Determined by GC/MS.

Maintenance of Non-Certified Values: NIST will monitor this material to the end of its period of validity. If substantive technical changes occur that affect the non-certified values during this period, NIST will update this Appendix and notify registered users. SRM users can register online from a link available on the NIST SRM website or fill out the user registration form that is supplied with the SRM. Registration will facilitate notification. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (<https://www.nist.gov/srm>).

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APPENDIX B

Development of SRM 3275 was a collaboration between NIST and the National Institutes of Health (NIH), Office of Dietary Supplements (ODS).

Source and Preparation: The SRM consists of three individual fish oils: Part 3275-1, a concentrate high in DHA; Part 3275-2, an anchovy oil high in EPA and DHA; and Part 3275-3, a concentrate containing 60 % long-chain omega-3 fatty acids (Ocean Nutrition, Dartmouth, NS, Canada). Mixed natural tocopherols at a minimum mass fraction of 1 mg/g were added as an antioxidant to all three oils by Ocean Nutrition. The oils were ampouled individually in amber glass ampoules that were flushed with argon. Each ampoule contains nominally 1.2 mL. The materials were stored at 4 °C following ampouling.

Analytical Approach for Determination of Fatty Acids: Value assignment of the concentrations of fatty acids in SRM 3275 was based on the combination of measurements made using two independent analytical methods at NIST: gas chromatography with flame ionization detection (GC-FID) and GC with mass spectrometric detection (GC/MS) as described below.

For GC-FID, two 0.5 g test portions from each of 10 ampoules of the three oils comprising SRM 3275 were combined with an internal standard solution containing octacosanoic acid and myristic-*d*₂₇ acid. MethPrep II [0.1 mol/L methanolic (*m*-trifluoromethylphenyl)trimethylammonium hydroxide, Alltech, Deerfield, IL] and 2,2,4-trimethylpentane were added stepwise for conversion of fatty acids to their methyl esters (FAMES) prior to analysis by GC-FID using a 0.24 mm × 100 m nonbonded biscyanopropyl polysiloxane fused silica capillary column. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the fatty acids in the SRM. A single internal standard solution was used for the calibrants and samples. Calculations are based on average response factors for the calibrants.

For GC/MS, two 0.5 g test portions from each of 10 ampoules of the three oils comprising SRM 3275 were combined with an internal standard solution containing octacosanoic acid and myristic-*d*₂₇ acid. A two-step process employing methanolic sodium hydroxide and boron trifluoride was used to convert the fatty acids to FAMES. GC/MS was performed using a 0.25 mm × 60 m fused silica capillary column containing a 50 % cyanopropyl + 50 % phenylpolysiloxane (mole fraction) phase. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the fatty acids in the SRM. A single internal standard solution was used for the calibrants and samples. Calculations are based on average response factors for the calibrants.

Homogeneity Assessment: The homogeneity of fatty acids was assessed at NIST using the methods and test portion sizes described above; analysis of variance did not show statistically significant heterogeneity.

Value Assignment: For calculation of assigned values, the means of the available individual sets of data were averaged.

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