



# Certificate of Analysis

## Standard Reference Material<sup>®</sup> 3275

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

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. 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 Mass Fraction Values:** The certified mass fraction values for selected 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].

**Reference Mass Fraction Values:** Reference mass fraction values for additional fatty acids are provided in Table 2. A NIST reference value is a noncertified value that 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].

**Expiration of Certification:** The certification of **SRM 3275** is valid, within the measurement uncertainty specified, until **31 March 2019**, provided the SRM is handled and stored in accordance with instructions given in this certificate (see “Instructions for Handling, 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 or register online) will facilitate notification.

Overall direction and coordination of the technical measurements leading to the certification of this SRM were performed by L.C. Sander, K.E. Sharpless, and S.A. Wise of the NIST Chemical Sciences Division and M.M. Schantz formerly of NIST.

Acquisition of the material was coordinated by M.M. Schantz.

Analytical measurements at NIST were performed by M.M. Schantz and C. Luvonga formerly of NIST.

The development of SRM 3275 was a collaboration between NIST and the National Institutes of Health (NIH), Office of Dietary Supplements (ODS). Technical consultation was provided by J. Betz of NIH-ODS.

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

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## INSTRUCTIONS FOR HANDLING, STORAGE, AND USE

**Handling:** 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.

## SOURCE, PREPARATION, AND ANALYSIS<sup>(1)</sup>

**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*<sub>27</sub> 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*<sub>27</sub> 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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<sup>(1)</sup> 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.

Table 1. Certified Mass Fraction Values for Fatty Acids as FAMES<sup>(a,b)</sup>

	Mass Fraction (mg/g)		
	Part 3275-1	Part 3275-2	Part 3275-3
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	

<sup>(a)</sup> 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  $U = ku_c$  where  $u_c$  is intended to represent, at the level of one standard deviation, the effects of the combined components of uncertainty, and where the coverage factor  $k = 2$  for a 95 % confidence interval (approximately) for each analyte.

<sup>(b)</sup> The measurand is the mass fraction for the fatty acids listed as FAMES. The certified values are metrologically traceable to the SI derived unit for mass fraction, expressed as milligrams per gram.

Table 2. Reference Mass Fraction Values for Fatty Acids as FAMES<sup>(a,b)</sup>

	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 <sup>(c)</sup>		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	1.61 ± 0.11		2.57			
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	

<sup>(a)</sup> Each reference 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.

<sup>(b)</sup> The measurand is the mass fraction for the fatty acids listed as FAMES. The reference values as determined by the methods indicated are metrologically traceable to the SI derived unit for mass fraction, expressed as milligrams per gram.

<sup>(c)</sup> Determined by GC/MS.

## REFERENCES

- [1] May, W.; Parris, R.; Beck II, 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/publications.cfm> (accessed June 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 (JCGM) (2008); available at [http://www.bipm.org/utis/common/documents/jcgm/JCGM\\_100\\_2008\\_E.pdf](http://www.bipm.org/utis/common/documents/jcgm/JCGM_100_2008_E.pdf) (accessed June 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 June 2016).
- [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 [http://www.bipm.org/utis/common/documents/jcgm/JCGM\\_101\\_2008\\_E.pdf](http://www.bipm.org/utis/common/documents/jcgm/JCGM_101_2008_E.pdf) (accessed June 2016).
- [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 <http://www.physics.nist.gov/Pubs> (accessed June 2016).

**Certificate Revision History:** **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).

*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](mailto:srminfo@nist.gov); or via the Internet at <http://www.nist.gov/srm>.*