



# Certificate of Analysis

## Standard Reference Material® 3274

### Botanical Oils Containing Omega-3 and Omega-6 Fatty Acids

This Standard Reference Material (SRM) is intended primarily for use in validating analytical methods for the determination of fatty acids in botanical oils and similar matrices. This SRM can also be used for quality assurance when assigning values to in-house reference materials. A unit of SRM 3274 consists of a total of four ampoules, one each of four seed oils: (3274-1 Borage [*Borago officinalis*], 3274-2 Evening Primrose [*Oenothera biennis*], 3274-3 Flax [*Linum usitatissimum*], and 3274-4 Perilla [*Perilla frutescens*]). Each ampoule contains approximately 1.2 mL of oil under argon.

**Certified Mass Fraction Values:** 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 mass fraction values of selected fatty acids are provided in bold typeface in Table 1. Values were derived from the combination of results provided by two or three analytical methods employed at NIST. The certified values in this material are the equally weighted means of the individual sets of data; the associated uncertainties are expanded uncertainties at the 95 % level of confidence [2,3]. Values are reported on an “as-received” basis in mass fraction units [4].

**Reference Mass Fraction Values:** Reference values are noncertified values that are the best estimate of the true values based on available data; however, the values do not meet the NIST criteria for certification and are 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 [1]. Reference mass fraction values for additional fatty acids are provided in normal typeface in Table 1.

**Expiration of Certification:** The certification of **SRM 3274** is valid, within the measurement uncertainty specified, until **28 February 2021**, 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 or register online) will facilitate notification.

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

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

Acquisition and preparation of the material was coordinated by K.E. Sharpless. Material preparation prior to ampouling was performed by M.C. Tims and R.J. Case of the NIST Chemical Sciences Division.

The development of SRM 3274 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.

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

Carlos A. Gonzalez, Chief  
Chemical Sciences Division

Robert L. Watters, Jr., Director  
Office of Reference Materials

Gaithersburg, MD 20899  
Certificate Issue Date: 25 June 2015  
Certificate Revision History on Page 5

## NOTICE AND WARNING TO USERS

WARNING: FOR LABORATORY USE ONLY; NOT FOR HUMAN CONSUMPTION.

## INSTRUCTIONS FOR STORAGE AND USE

**Storage:** The material should be stored under refrigeration (0 °C to 4 °C), in an unopened ampoule, until required for use.

**Use:** Prior to removal of a test portion for analysis, the contents of an ampoule of material should be allowed to warm to room temperature and mixed thoroughly. (Solids that have precipitated during refrigeration should redissolve when the material reaches room temperature.) Test portions used for fatty acid analyses described below were 0.5 g. For certified values to be valid, test portions of at least 0.5 g should be used.

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

**Material Acquisition and Preparation:** Borage oil, evening primrose oil, flaxseed oil, and perilla oil from single lots were obtained from Arista Industries, Inc., (Wilton, CT). Individual oils were combined with *tert*-butylhydroquinone (TBHQ), an antioxidant, to yield a final TBHQ concentration of approximately 190 mg/L. To facilitate dissolution of the TBHQ, the mixture was stirred overnight, at room temperature, using a magnetic stir bar. SRM 3274 was ampouled under argon with each ampoule containing approximately 1.2 mL of oil.

**Analytical Approach for Determination of Fatty Acids:** Value assignment of the concentrations of the fatty acids in SRM 3274 was based on the combination of measurements from three different analytical methods at NIST: a combination of two independent sample preparation methods and two gas chromatography (GC) methods with different detection (i.e., flame ionization detection [FID] and mass spectrometry [MS] as described below).

## NIST ANALYSES FOR FATTY ACIDS

Fatty acids were measured by using combinations of two sample preparation methods and two GC methods with FID or MS detection. Six independently prepared calibrants were used for each of the methods. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the fatty acids in the SRM. A single internal standard solution containing stearic-*d*<sub>35</sub> acid and myristic-*d*<sub>27</sub> acid was used for the calibrants and samples. Calculations are based on average response factors for the calibrants.

**Sample Preparation Method 1:** Two 0.5 g test portions from each of ten ampoules were combined with the internal standard solution. Approximately 0.5 mL of MethPrep II (0.1 mol/L methanolic [*m*-trifluoromethylphenyl]-trimethylammonium hydroxide, Alltech; Deerfield, IL) was added, samples were mixed for 1 min, and allowed to stand for at least 30 min prior to addition of toluene and analysis by using GC-FID.

**Sample Preparation Method 2:** Two 0.5 g test portions from one ampoule and single 0.5 g test portions from each of another nine ampoules were combined with the internal standard. A two-step process involving methanolic sodium hydroxide and boron trifluoride was used to convert the fatty acids to their methyl esters. Methyl esters were extracted into hexane three times, and the combined hexane portions were concentrated to approximately 1 mL. Following the addition of toluene, samples were analyzed by using GC/MS and GC-FID.

**GC with Flame Ionization Detection:** GC-FID was performed using a 0.25 mm × 100 m biscyanopropyl polysiloxane fused silica capillary column. A typical separation is provided in Appendix A.

**GC with Mass Spectrometric Detection:** GC/MS was performed using a 0.25 mm × 60 m cyanopropyl and phenylpolysiloxane fused silica capillary column. The MS was operated in the scan mode (70 amu to 400 amu).

**Homogeneity Assessment:** The homogeneity of fatty acids was assessed at NIST by using the GC-FID method described above. An analysis of variance did not show inhomogeneity for a 0.5 g sample.

**Value Assignment:** Each value is the equally weighted average of the method means available for that analyte. Each method mean is the mean of the measurements for that analyte. Certified values are means of either two or three method means. A reference value may be based on one to three method means. The uncertainty of each value is estimated following the method in the ISO/JCGM Guide [2].

---

<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 (in Bold) and Reference (in Normal Typeface) Mass Fraction Values for Selected Fatty Acids (as Triglycerides) in SRM 3274<sup>(a)</sup>

	Mass Fraction (mg/g)							
	3274-1		3274-2		3274-3		3274-4	
	Borage <sup>(b)</sup>	(k)	Evening Primrose <sup>(c)</sup>	(k)	Flax <sup>(d)</sup>	(k)	Perilla <sup>(e)</sup>	(k)
Octanoic Acid (C8:0) (Caprylic Acid)			0.053 ± 0.010	(3.18)			0.021 ± 0.002	(2.57)
Decanoic Acid (C10:0) (Capric Acid)							0.020 ± 0.011	(12.71)
Dodecanoic Acid (C12:0) (Lauric Acid)					0.016 ± 0.001	(2.26)		
Tetradecanoic Acid (C14:0) (Myristic Acid)	0.62 ± 0.11	(2.01)	<b>0.363 ± 0.030</b>	<b>(2.23)</b>	<b>0.271 ± 0.008</b>	<b>(2.20)</b>	0.206 ± 0.025	(2.03)
Pentadecanoic Acid (C15:0)	<b>0.074 ± 0.008</b>	<b>(2.12)</b>	<b>0.099 ± 0.011</b>	<b>(2.57)</b>	<b>0.151 ± 0.016</b>	<b>(2.13)</b>	0.061 ± 0.009	(2.04)
Hexadecanoic Acid (C16:0) (Palmitic Acid)	<b>110 ± 12</b>	<b>(2.13)</b>	<b>58.2 ± 6.1</b>	<b>(2.04)</b>	<b>44.8 ± 5.0</b>	<b>(2.06)</b>	<b>56.4 ± 5.5</b>	<b>(2.07)</b>
(Z)-9-Hexadecenoic Acid (C16:1 n-7) (Palmitoleic Acid)	<b>1.77 ± 0.14</b>	<b>(2.45)</b>	<b>0.402 ± 0.043</b>	<b>(2.16)</b>	<b>0.383 ± 0.031</b>	<b>(2.36)</b>	<b>0.776 ± 0.081</b>	<b>(2.16)</b>
Heptadecanoic Acid (C17:0) (Margaric Acid)	0.500 ± 0.086	(2.00)	0.188 ± 0.008	(2.36)	0.212 ± 0.011	(2.26)	0.159 ± 0.040	(2.00)
Octadecanoic Acid (C18:0) (Stearic Acid)	33.1 ± 4.0	(2.03)	<b>18.30 ± 0.838</b>	<b>(2.36)</b>	<b>30.4 ± 2.4</b>	<b>(2.31)</b>	<b>20.9 ± 1.1</b>	<b>(2.57)</b>
(Z)-9-Octadecenoic Acid (C18:1 n-9) (Oleic Acid)	<b>148.7 ± 8.7</b>	<b>(2.09)</b>	<b>68.9 ± 3.7</b>	<b>(2.78)</b>	<b>165.7 ± 6.2</b>	<b>(2.45)</b>	<b>166.8 ± 7.8</b>	<b>(2.36)</b>
(E)-9-Octadecenoic Acid (t-C18:1 n-9) (Elaidic Acid)	0.117 ± 0.020	(2.14)						
(Z)-11-Octadecenoic Acid (C18:1 n-7) (Vaccenic Acid)	<b>5.76 ± 0.18</b>	<b>(2.36)</b>	<b>5.95 ± 0.37</b>	<b>(2.18)</b>	5.61 ± 0.16	(2.26)	7.89 ± 0.22	(2.23)
(Z,Z)-9,12-Octadecadienoic Acid (C18:2 n-6) (Linoleic Acid)	<b>374 ± 35</b>	<b>(2.11)</b>	<b>742 ± 24</b>	<b>(2.57)</b>	<b>171 ± 11</b>	<b>(2.23)</b>	<b>160 ± 14</b>	<b>(2.02)</b>
(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3 n-3) ( $\alpha$ -Linolenic Acid)	1.97 ± 0.11	(2.57)	1.60 ± 0.12	(2.57)	<b>579 ± 30</b>	<b>(2.31)</b>	<b>629 ± 28</b>	<b>(2.78)</b>
(Z,Z,Z)-6,9,12-Octadecatrienoic Acid (C18:3 n-6) ( $\gamma$ -Linolenic Acid)	<b>251 ± 24</b>	<b>(2.06)</b>	<b>99.9 ± 4.1</b>	<b>(2.31)</b>	1.55 ± 0.25	(2.16)	2.08 ± 0.48	(2.09)

Table 1 (continued). Certified (in Bold) and Reference (in Normal Typeface) Mass Fraction Values for Selected Fatty Acids (as Triglycerides) in SRM 3274<sup>(a)</sup>

	Mass Fraction (mg/g)							
	3274-1		3274-2		3274-3		3274-4	
	Borage <sup>(b)</sup>	( <i>k</i> )	Evening Primrose <sup>(c)</sup>	( <i>k</i> )	Flax <sup>(d)</sup>	( <i>k</i> )	Perilla <sup>(e)</sup>	( <i>k</i> )
Eicosanoic Acid (C20:0) (Arachidic Acid)	2.13 ± 0.46	(2.00)	2.71 ± 0.37	(2.18)	1.04 ± 0.15	(2.02)	1.21 ± 0.26	(2.00)
( <i>Z</i> )-11-Eicosenoic Acid (C20:1 n-9) (Gondoic Acid)			<b>1.84 ± 0.12</b>	<b>(2.36)</b>				
( <i>Z,Z,Z,Z</i> )-5,8,11,14-Eicosatetraenoic Acid (C20:4 n-6) (Arachidonic Acid)			0.022 ± 0.002	(2.78)	<b>0.633 ± 0.029</b>	<b>(2.23)</b>		
Heneicosanoic Acid (C21:0)	2.36 ± 0.32	(2.00)	0.132 ± 0.029	(2.31)			0.083 ± 0.004	(2.09)
Docosanoic Acid (C22:0) (Behenic Acid)	<b>1.509 ± 0.070</b>	<b>(2.26)</b>	0.91 ± 0.25	(2.01)	0.62 ± 0.13	(2.00)	0.118 ± 0.004	(2.09)
Tetracosanoic Acid (C24:0) (Lignoceric Acid)	0.334 ± 0.074	(2.00)	0.369 ± 0.060	(2.05)	0.308 ± 0.059	(2.01)	0.096 ± 0.022	(2.00)
( <i>Z</i> )-15-Tetracosenoic Acid (C24:1) (Nervonic Acid)	<b>7.80 ± 0.61</b>	<b>(2.78)</b>					<b>0.084 ± 0.003</b>	<b>(2.11)</b>

<sup>(a)</sup> Each certified and reference mass fraction value is an equally weighted mean of the results provided by NIST. The uncertainty in each 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-laboratory and within-laboratory components of uncertainty. The coverage factor ( $k$ ) is determined from the Student's  $t$ -distribution corresponding to the appropriate associated degrees of freedom and approximately 95 % confidence for each analyte. All certified and reference values are based on results from three methods (GC-FID using sample preparation methods 1 and 2 [GC-FID1 and GC-FID2, respectively] and GC/MS) except as indicated below. The measurands for the certified values are mass fractions reported in bold in Table 1; the measurands for the reference values are the mass fractions in Table 1, normal font, and are as determined by the methods indicated above. The values are metrologically traceable to the SI unit of mass.

<sup>(b)</sup> For SRM 3274-1 Borage Oil, heneicosanoic acid was determined by using GC-FID1 and GC-FID2; vaccenic acid was determined by using GC-FID2 and GC/MS; behenic acid was determined by using GC-FID1 and GC/MS; and  $\alpha$ -linolenic and elaidic acid were determined by using GC-FID1.

<sup>(c)</sup> For SRM 3274-2 Evening Primrose Oil, vaccenic acid and gondoic acid were determined by using GC-FID1 and GC/MS; caprylic acid and margaric acid were determined by using GC/MS;  $\alpha$ -linolenic acid was determined using GC-FID1; and arachidonic acid was determined by using GC-FID2.

<sup>(d)</sup> For SRM 3274-3 Flax Oil, arachidonic acid was determined by using GC-FID1 and GC-FID2; lauric acid was determined by using GC-FID2; and margaric acid and vaccenic acid were determined by using GC/MS.

<sup>(e)</sup> For SRM 3274-4 Perilla Oil, margaric acid was determined by using GC-FID2 and GC/MS; nervonic acid was determined by using GC-FID1 and GC-FID2; heneicosanoic acid and behenic acid were determined by using GC-FID1; caprylic acid and capric acid were determined by using GC-FID2; and vaccenic acid was determined by using 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 2015).
- [2] JCGM 100:2008; *Evaluation of Measurement Data - Guide to the Expression of Uncertainty in Measurement*; (ISO GUM 1995 with Minor Corrections), Joint Committee for Guides in Metrology (JCGM) (2008); available at [http://www.bipm.org/utls/common/documents/jcgm/JCGM\\_100\\_2008\\_E.pdf](http://www.bipm.org/utls/common/documents/jcgm/JCGM_100_2008_E.pdf) (accessed June 2015); 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 2015).
- [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.physics.nist.gov/Pubs> (accessed June 2015).

**Certificate Revision History:** 25 June 2015 (Change expiration date; editorial changes); 02 January 2013 (Reference mass fraction values updated for  $\alpha$ -linolenic acid in SRM 3274-1 and SRM 3274-2;  $k$  values added to Table 1; editorial changes); 27 January 2009 (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>.*

APPENDIX A

Typical chromatogram from the analysis of SRM 3274 using GC-FID on an SP 2560 (Supelco, Bellefonte, PA). The column was held isothermally at 100 °C for 4 min and then temperature programmed at 2.5 °C per min to 240 °C for 50 min. The injection port and FID were maintained at 240 °C. All injections were done in the split mode (1 µL) with helium as a carrier gas at a constant flow rate of 1 mL/min.



