National Institute of Standards & Technology

Report of Investigation

Reference Material 8037

Krill Oil

Reference Material (RM) 8037 is intended primarily for use in evaluation of analytical methods for determining fatty acids in fish oils, dietary supplements, and similar materials. This RM provides a common matrix to those in the natural products community who wish to conduct research studies. A unit of RM 8037 consists of three screw-top tins, each containing approximately 4.5 mL of krill oil.

The development of RM 8037 was a collaboration between the National Institute of Standards and Technology (NIST) and the National Institutes of Health Office of Dietary Supplements (NIH-ODS).

Reference Mass Fraction Values of Fatty Acids: Reference mass fraction values for fatty acids in RM 8037 are provided in Table 1. 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 an associated uncertainty 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. The reference mass fraction values were derived from results reported by NIST and/or collaborating laboratories. Values are reported on an as-received basis in mass fraction units of free fatty acids in RM 8037 [2].

Expiration of Value Assignments: RM 8037 is valid, within the measurement uncertainty specified, until **31 December 2029,** provided the RM is handled and stored in accordance with instructions given in this Report of Investigation (see "Instructions for Storage and Use"). This report is nullified if the RM is damaged, contaminated, or otherwise modified.

Maintenance of RM Value Assignment: NIST will monitor this RM over the period of its value assignment. If substantive technical changes occur that affect the value assignments before the expiration of this report, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Coordination of the technical measurements leading to the value assignment of this RM was performed by C.A. Rimmer and B.J. Place of the NIST Chemical Sciences Division.

Technical consultation was provided by J.M. Betz (NIH-ODS). Acquisition and preparation of the material was coordinated by M.M. Schantz formerly of NIST.

Coordination of the distribution of materials and reporting of measurement results for an interlaboratory comparison exercise were performed by M.M. Phillips and L.J. Wood of the NIST Chemical Sciences Division and by W. Koshute and B. St. Amant of the Grocery Manufacturers Association (GMA).

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

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

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

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Report Issue Date: 20 April 2020

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Analysts at the following laboratories performed measurements that contributed to the value assignment of fatty acids in RM 8037 Krill Oil as part of a GMA Food Industry Analytical Chemists Share Group (FIACSG) interlaboratory comparison exercise: ConAgra Foods (Omaha, NE); Covance Inc.(Madison, WI); Covance Pte. Ltd. (Asia); The Synergy (Singapore); Covance Inc. (Harrogate, UK); Del Monte Foods (Walnut Creek, CA); Eurofins Central Analytical Laboratories (Metairie, LA); Eurofins Scientific (Des Moines, IA); General Mills Inc. (Golden Valley, MN); Hormel Foods (Austin, MN); Krueger Food Labs (Chelmsford, MA); Land O' Lakes (Arden Hills, MN); Mereiux NutriSciences Brasil (Sao Paolo, Brazil); Nestlé Brasil Ltda. (Sao Paolo, Brazil); Nestle Quality Assurance Center (Dublin, OH); Schwan Food Company (Salina, KS); The J.M. Smucker Co. (Orrville, OH); and The National Food Laboratory (Livermore, CA).

NOTICE TO USERS: RM 8037 IS INTENDED FOR RESEARCH USE; NOT FOR HUMAN OR ANIMAL CONSUMPTION.

INSTRUCTIONS FOR STORAGE AND USE

Storage: The RM should be stored frozen (–20 °C or colder), in original unopened tins, until required for use. Value assignment does not apply to contents of previously opened and stored tins as the stability of fatty acids has not been investigated in previously opened tins.

Use: Before use, the contents of the tin should be allowed to warm to room temperature and mixed thoroughly. To relate analytical determinations to the values in this Report of Investigation, a minimum test portion size of 0.2 g should be used to ensure valid results. Results obtained in analyses should include their own estimates of uncertainty and can be compared to the non-certified or reference values using procedures described in reference 3.

SOURCE, PREPARATION, AND ANALYSIS[(1)]

Source and Preparation: The RM is a krill oil prepared by a commercial manufacturer. A 5 kg container of krill oil was received at NIST from a commercial manufacturer of krill oil. The oil was warmed to room temperature and gently rotated to mix the contents. Approximately 4.5 mL of krill oil was transferred into screw-top tins, then stored at –20 °C.

Analytical Approach for Determination of Fatty Acids: Value assignment of the mass fractions of fatty acids in RM 8037 was based on the combination of measurements made using gas chromatography with flame ionization detection (GC-FID) at NIST and collaborating laboratories, where appropriate.

GC-FID measurements at NIST: Mass fractions of free fatty acids were determined from duplicate, nominal 0.2 g test portions from 10 screw-top tins of RM 8037. The test portions of krill oil were weighed into 15 mL centrifuge tubes with Teflon caps. One milliliter of chloroform was added to each tube volumetrically and 1 mL of internal standard solution containing myristic acid-d_{27} and palmitic acid-d_{31} was added gravimetrically. Additions of 1.5 mL of 1.0 mol/L NaOH (in methanol); 2.0 mL of 14 % (volume fraction) boron trifluoride (BF_3 in methanol); 3 mL of 40 mg/L BHT (in hexane); and 5 mL of saturated NaCl (aq.) were added and, after each addition, the samples were blanketed with N_2, capped, and heated in a dry bath. A portion of the hexane/BHT layer was then transferred to an autosampler vial for GC-FID analysis. Analysis was by GC-FID using a 0.32 mm × 30 m FAMEWAX column with a 0.25 µm film. Calibrants were prepared gravimetrically from SRM 2377 *Fatty Acid Methyl Esters in 2,2,4-Trimethylpentane*, at levels intended to approximate the levels of fatty acids in RM 8037. A single internal standard solution was used for the calibrants and samples. Calculations are based on average response factors for the calibrants.

Collaborating Laboratories' Analyses: The GMA FIACSG laboratories were asked to use their usual methods to make single measurements on test portions taken from each of two tins of RM 8037 for measurements of fatty acids. Because of the variability among data provided by laboratories participating in an interlaboratory comparison exercise, the median of laboratory means is used, with the uncertainty estimated using the median absolute deviation (MADe) [4]. The median of the collaborating laboratories' means was combined with NIST data for calculation of reference values of fatty acids, where appropriate, with the uncertainty estimated using the standard error of the mean of laboratory means. The methods used by collaborating laboratories were all reported as utilizing a sample hydrolysis and derivatization step followed by analysis using GC-FID.

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(1) Certain commercial equipment, instruments, or materials are identified in this report of investigation in order to specify adequately 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.

Homogeneity Assessment: The homogeneity of fatty acids was assessed at NIST using the methods and test portion sizes described above. Analysis of variance showed possible statistically significant heterogeneity for (Z)-9-Tetradecenoic Acid (C14:1), Octadecanoic Acid (C18:0), (Z)-9-Octadecenoic Acid (C18:1 n-9), (Z)-11-Octadecenoic Acid (C18:1 n-7), and (Z,Z)-9,12-Octadecadienoic Acid (C18:2 n-6), and the uncertainties for these values incorporate an additional component for possible heterogeneity.

Value Assignment: For calculation of assigned values for analytes that were measured only at NIST, the mean values from NIST results were used. The collaborating laboratories reported the individual results for each of their analyses for a given analyte. The mean of each laboratory's results was then determined. For calculation of assigned values for analytes that were measured only by the GMA FIACSG laboratories, the median of the laboratory means was used. For analytes that were also measured by NIST, the mean of the individual sets of NIST data were averaged with the median of the individual GMA FIACSG laboratory means.

Reference Mass Fraction Values for Fatty Acids as Free Fatty Acids: Each reference mass fraction value is the mean result of a NIST analysis using a single method or the mean from the combination of NIST results with the median of the mean results provided by collaborating laboratories where appropriate. Values are expressed as $x \pm U_{95\%}(x)$, where x is the estimated value and $U_{95\%}(x)$ is the expanded uncertainty of the value. The method-specific value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with about a 95 % confidence [5-7]. The measurands are the mass fractions of fatty acids in krill oil listed in Table 1, on an as-received basis, as determined by the method indicated. Metrological traceability is to the measurement processes and standards used by NIST and collaborating laboratories.

Table 1. Reference Mass Fraction Values for Fatty Acids (as Free Fatty Acids) in RM 8037

Analyte	Common Name	Mass Fraction (mg/g)
Octanoic Acid (C8:0)[a]	Caprylic Acid	0.061 ± 0.008
Decanoic Acid (C10:0)[a]	Capric Acid	3.15 ± 0.23
Dodecanoic Acid (C12:0)[a,b]	Lauric Acid	1.35 ± 0.13
Tetradecanoic Acid (C14:0)[a,b]	Myristic Acid	60 ± 11
(Z)-9-Tetradecenoic Acid (C14:1)[a,b]	Myristoleic Acid	1.05 ± 0.10
Hexadecanoic Acid (C16:0)[a,b]	Palmitic Acid	128.5 ± 7.8
(Z)-9-Hexadecenoic Acid (C16:1 n-7)[a,b]	Palmitoleic Acid	33.8 ± 2.9
Octadecanoic Acid (C18:0)[a,b]	Stearic Acid	6.9 ± 1.3
(Z)-9-Octadecenoic Acid (C18:1 n-9)[a,b]	Oleic Acid	60.8 ± 4.9
(Z)-11-Octadecenoic Acid (C18:1 n-7)[a,b]	Vaccenic Acid	39.7 ± 2.4
(Z,Z)-9,12-Octadecadienoic Acid (C18:2 n-6)[a,b]	Linoleic Acid	16.3 ± 2.5
(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3 n-3)[a,b]	α-Linolenic Acid	8.50 ± 0.98
(Z,Z,Z)-6,9,12-Octadecatrienoic Acid (C18:3 n-6)[a,b]	γ-Linolenic Acid	1.17 ± 0.29
Docosanoic Acid (C22:0)[a,b]	Behenic Acid	0.31 ± 0.09
(Z)-11-Eicosenoic Acid (C20:1 n-9)[a]	Gondoic Acid	3.88 ± 0.16
(Z,Z,Z,Z)-5,8,11,14-Eicosatetraenoic Acid (C20:4 n-6)[a,b]	Arachidonic Acid	2.49 ± 0.10
(Z)-13-Docosenoic Acid (C22:1 n-9)[a,b]	Erucic Acid	3.50 ± 0.51
(Z,Z,Z,Z,Z)-5,8,11,14,17-Eicosapentaenoic Acid[a,b]	EPA	118.8 ± 3.8
(Z,Z,Z,Z,Z)-7,10,13,16,19-Docosapentaenoic Acid (C22:5 n-3)[a,b]	DPA	3.28 ± 0.14
(Z,Z,Z,Z,Z,Z)-4,7,10,13,16,19-Docosahexaenoic Acid (C22:6 n-3)[a,b]	DHA	74 ± 11
Tetracosanoic Acid (C24:0)[a]	Lignoceric Acid	0.430 ± 0.042
(Z)-15-Tetracosenoic Acid (C24:1 n-9)[a]	Nervonic Acid	1.08 ± 0.14

[a] NIST GC-FID.
[b] Collaborating Laboratories. Reported methods included hydrolysis and derivatization followed by GC-FID.

REFERENCES

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[7] Efron, B.; Ribshirani, R.J.; *An Introduction to the Bootstrap;* Chapman & Hall, London, UK (1993).

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