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Reference Material 8183

Omega-3 and Omega-6 Fatty Acids in Botanical Oils

REFERENCE MATERIAL INFORMATION SHEET

Purpose: This Reference Material (RM) is intended primarily for use in evaluating analytical methods and chromatographic instrumentation for the determination of fatty acids in botanical oils and similar matrices. A unit of RM 8183 consists of a total of four ampoules, one each of four seed oils: (8183-1 Borage [*Borago officinalis*], 8183-2 Evening Primrose [*Oenothera biennis*], 8183-3 Flax [*Linium usitatissimum*], and 8183-4 Perilla [*Perilla frutescens*]). Each ampoule contains approximately 1.2 mL of oil under argon.

Due to the original NIST fatty acid determinations in SRM 3274 not being sufficiently traceable to the International System of Units (SI) through use of SRM 2377 Fatty Acid Methyl Esters in 2,2,4-Trimethylpentane as a calibrant, SRM 3274 no longer meets international quality standards (ISO 17034) for serving as a certified reference material. However, the material continues to meet ISO 17034 standards as a reference material and is thus being offered as RM 8183.

Non-Certified Values: NIST non-certified values do not meet the NIST criteria for certification [1] and are the best estimates of the true values based on available data. The values are provided with an uncertainty that may reflect only measurement reproducibility, may not include all sources of uncertainty, and/or may reflect a lack of sufficient statistical agreement among multiple analytical methods.

Non-certified mass fraction values for analytes in RM 8183, reported on an as-received basis, are provided in Table 1. Values are expressed as $x \pm U_{95\%}(x)$, where x is the non-certified value and $U_{95\%}(x)$ is the expanded uncertainty of the non-certified value. The method-specific value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with 95 % confidence. To propagate this uncertainty, the non-certified value should be treated as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [2–4]. The measurands are the total mass fraction of each analyte in Table 1. Non-certified values are traceable to the measurement processes and standards used by NIST, (expressed as milligrams per gram (mg/g)) as realized by the methods used [2].

Period of Validity: The non-certified values are valid within the measurement uncertainty specified until **28 February 2031**. The value assignments are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of Non-Certified Value: 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 Reference Material Information Sheet. Before making use of any of the values delivered by this material, users should obtain the most recent version of this documentation, available free of charge through the https://www.nist.gov/srm website.

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Chemical Sciences Division	Office of Reference Materials

Table 1. Non-Certified Mass Fraction Values for Selected Fatty Acids (as Triglycerides) in RM 8183(a)

Mass Fraction
(mg/g)

	8183-1 Borage(b)	8183-2 Evening Primrose(c)	8183-3 Flax(d)	8183-4 Perilla(e)
Octanoic Acid (C8:0) (Caprylic Acid)		0.053 ± 0.010		0.021 ± 0.002
Decanoic Acid (C10:0) (Capric Acid)				0.020 ± 0.011
Dodecanoic Acid (C12:0) (Lauric Acid)			0.016 ± 0.001	
Tetradecanoic Acid (C14:0) (Myristic Acid)	0.62 ± 0.11	0.363 ± 0.030	0.271 ± 0.008	0.206 ± 0.025
Pentadecanoic Acid (C15:0)	0.074 ± 0.008	0.099 ± 0.011	0.151 ± 0.016	0.061 ± 0.009
Hexadecanoic Acid (C16:0) (Palmitic Acid)	110 ± 12	58.2 ± 6.1	44.8 ± 5.0	56.4 ± 5.5
(Z)-9-Hexadecenoic Acid (C16:1 n-7) (Palmitoleic Acid)	1.77 ± 0.14	0.402 ± 0.043	0.383 ± 0.031	0.776 ± 0.081
Heptadecanoic Acid (C17:0) (Margaric Acid)	0.500 ± 0.086	0.188 ± 0.008	0.212 ± 0.011	0.159 ± 0.040
Octadecanoic Acid (C18:0) (Stearic Acid)	33.1 ± 4.0	18.30 ± 0.838	30.4 ± 2.4	20.9 ± 1.1
(Z)-9-Octadecenoic Acid (C18:1 n-9) (Oleic Acid)	148.7 ± 8.7	68.9 ± 3.7	165.7 ± 6.2	166.8 ± 7.8
(E)-9-Octadecenoic Acid (t-C18:1 n-9) (Elaidic Acid)	0.117 ± 0.020			
(Z)-11-Octadecenoic Acid (C18:1 n-7) (Vaccenic Acid)	5.76 ± 0.18	5.95 ± 0.37	5.61 ± 0.16	7.89 ± 0.22
(Z,Z)-9,12-Octadecadienoic Acid (C18:2 n-6) (Linoleic Acid)	374 ± 35	742 ± 24	171 ± 11	160 ± 14
(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3 n-3) (α-Linolenic Acid)	1.97 ± 0.11	1.60 ± 0.12	579 ± 30	629 ± 28
(Z,Z,Z)-6,9,12-Octadecatrienoic Acid (C18:3 n-6) (γ-Linolenic Acid)	251 ± 24	99.9 ± 4.1	1.55 ± 0.25	2.08 ± 0.48

Table 1 (continued). Non-Certified Mass Fraction Values for Selected Fatty Acids (as Triglycerides) in RM 8183[(a)]

	Mass Fraction (mg/g)			
	8183-1 Borage[(b)]	8183-2 Evening Primrose[(c)]	8183-3 Flax[(d)]	8183-4 Perilla[(e)]
Eicosanoic Acid (C20:0) (Arachidic Acid)	2.13 ± 0.46	2.71 ± 0.37	1.04 ± 0.15	1.21 ± 0.26
(Z)-11-Eicosenoic Acid (C20:1 n-9) (Gondoic Acid)		1.84 ± 0.12		
(Z,Z,Z,Z)-5,8,11,14-Eicosatetraenoic Acid (C20:4 n-6) (Arachidonic Acid)		0.022 ± 0.002	0.633 ± 0.029	
Heneicosanoic Acid (C21:0)	2.36 ± 0.32	0.132 ± 0.029		0.083 ± 0.004
Docosanoic Acid (C22:0) (Behenic Acid)	1.509 ± 0.070	0.91 ± 0.25	0.62 ± 0.13	0.118 ± 0.004
Tetracosanoic Acid (C24:0) (Lignoceric Acid)	0.334 ± 0.074	0.369 ± 0.060	0.308 ± 0.059	0.096 ± 0.022
(Z)-15-Tetracosenoic Acid (C24:1) (Nervonic Acid)	7.80 ± 0.61			0.084 ± 0.003

[(a)] Each non-certified 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 [2,3,5]. All values are based on results from NIST (GC-FID using sample preparation methods 1 and 2 [GC-FID1 and GC-FID2, respectively] and GC/MS) except as indicated below.

[(b)] For RM 8183-1 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 α-linolenic and elaidic acid were determined by using GC-FID1.

[(c)] For RM 8183-2 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; α-linolenic acid was determined using GC-FID1; and arachidonic acid was determined by using GC-FID2.

[(d)] For RM 8183-3 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.

[(e)] For RM 8183-4 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.

Safety: RM 8183 IS INTENDED FOR RESEARCH USE; NOT FOR HUMAN CONSUMPTION.

Storage: RM 8183 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 assigned values to be valid, test portions of at least 0.5 g should be used.

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. RM 8183 was ampouled under argon with each ampoule containing approximately 1.2 mL of oil.

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 (NIST SP) 260-136, 2021 edition; U.S. Government Printing Office: Washington, DC (2021); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2021.pdf (accessed Dec 2021).

[2] 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 Dec 2021).

[3] 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/publications/guides (accessed Dec 2021); 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 Dec 2021).

[4] 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/publications/guides (accessed Dec 2021).

[5] 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).

Users of this RM should ensure that the Reference Material Information Sheet 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.

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APPENDIX A

RESPONSIBILITIES

Coordination: C.A. Rimmer of the NIST Chemical Sciences Division.

The development of RM 8183 was a collaboration among the National Institute of Standards and Technology (NIST), the National Institutes of Health Office of Dietary Supplements (NIH-ODS), and the Food and Drug Administration Center for Drug Evaluation and Research (FDA CDER).

Analytical Measurements: M.M. Schantz formerly of the NIST Chemical Sciences Division. Stability measurements were conducted by Eurofins using GC-FID.

Statistical Analysis: J.H. Yen of the NIST Statistical Engineering Division.

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APPENDIX B

SOURCE, PREPARATION AND ANALYSIS

Analytical Approach for Determination of Fatty Acids: Value assignment of the mass fractions of the fatty acids in RM 8183 was based on the combination of measurements at NIST using gas chromatography (GC) with flame ionization detection (FID) or GC with mass spectrometry (MS), as described below.

Fatty acids were measured by using combinations of two sample preparation methods and two GC methods with FID, or GC with 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 RM. A single internal standard solution containing stearic-d_{35} acid and myristic-d_{27} 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 C.

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 mean of the method means available for that analyte. The uncertainty of each value is estimated following the method in the ISO/JCGM Guide [3].

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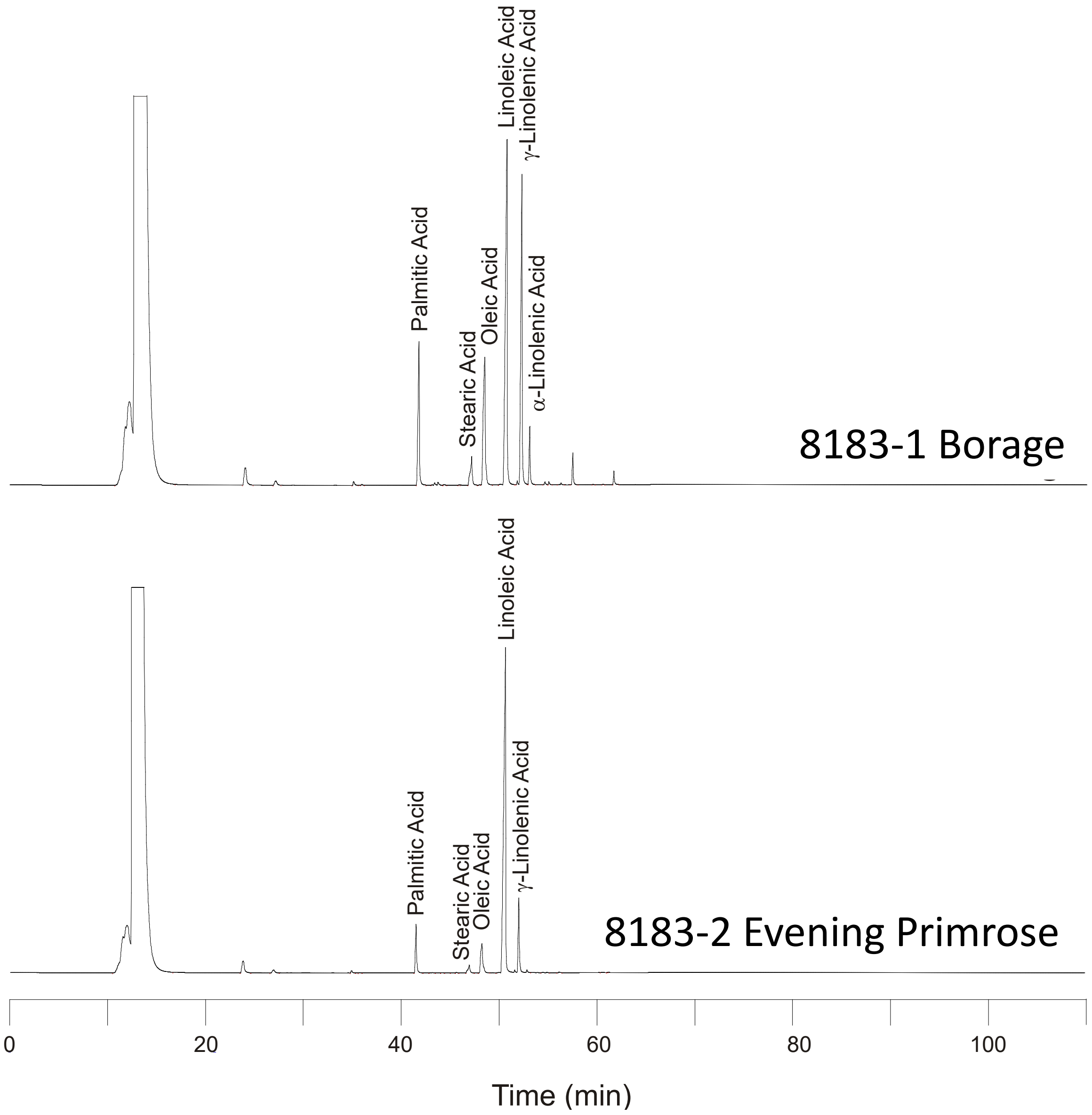
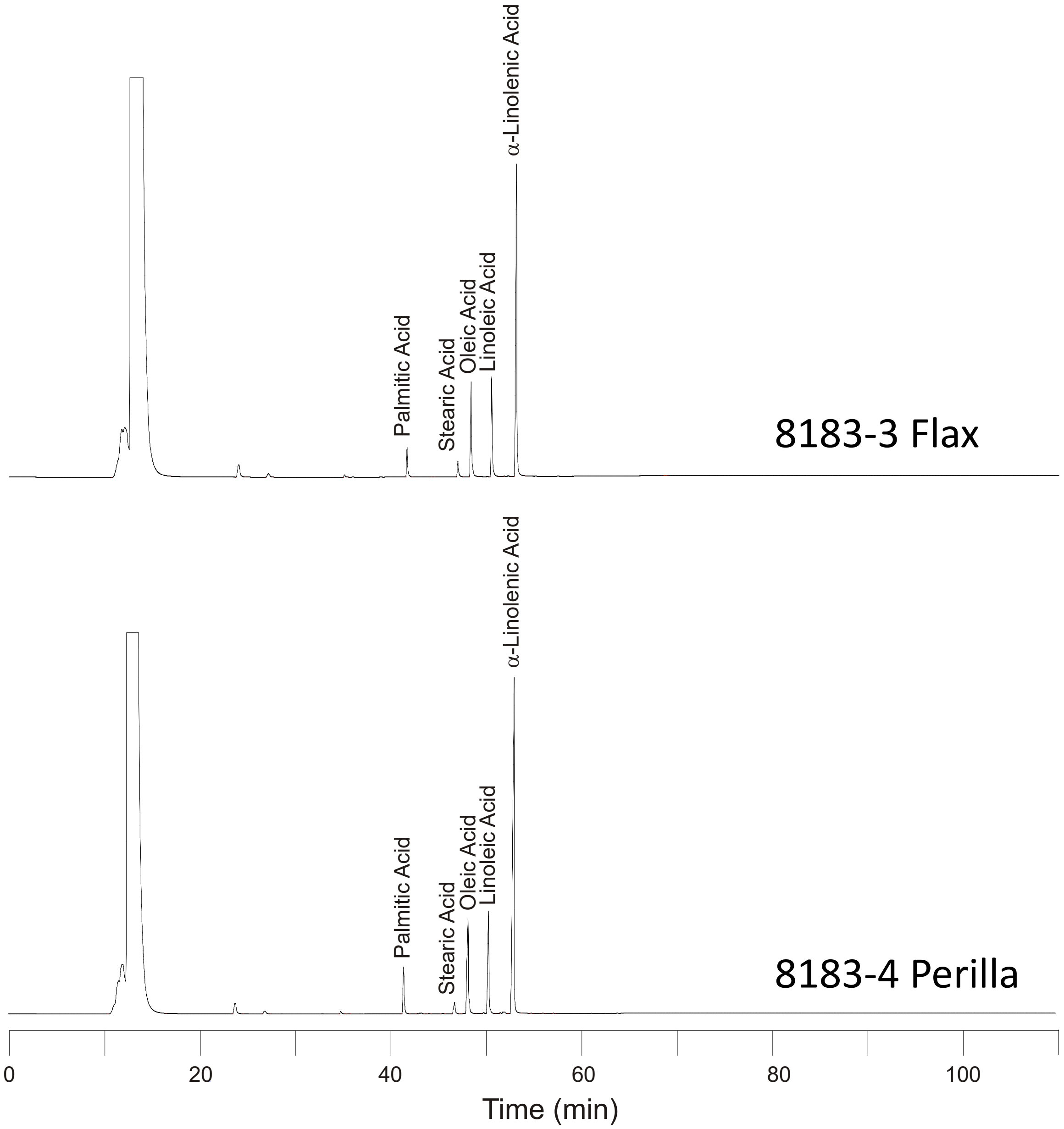
APPENDIX C

Typical chromatogram from the analysis of RM 8183 using GC-FID on an SP 2560 (Supelco, Bellefonte, PA). The column was held isothermally at 100 ºC for 4 min and then temperature programmed at 2.5 ºC per min to 240 ºC for 50 min. The injection port and FID were maintained at 240 ºC. 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.





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