



# Certificate of Analysis

## Standard Reference Material<sup>®</sup> 2772

### B100 Biodiesel (Soy-Based)

This Standard Reference Material (SRM) is a commercial 100 % biodiesel produced from soy and is intended for use in evaluating analytical methods for the determination of selected chemical and physical properties in pure biodiesel (B100). A unit of SRM 2772 consists of five 10-milliliter ampoules, each containing approximately 10 mL of biodiesel.

**Certified Values:** Certified mass fraction values for four fatty acid methyl esters and water are provided in Tables 1 and 2, respectively. Certified values for density at 20 °C and kinematic viscosity at 20 °C, 30 °C, and 40 °C are provided in Table 3. 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 values are based on the agreement of results obtained at NIST using one or more analytical techniques and additional results from the National Institute of Metrology, Standardization, and Industrial Quality (INMETRO), Brazil and Cannon Instrument Company (State College, PA) using different analytical techniques.

**Reference Values:** A reference mass fraction value for docosanoic acid methyl ester is provided in Table 4. Reference values for methanol and additional physical properties are provided in Tables 5 and 6, respectively. Reference values are noncertified values that are estimates of the true value; however, the values do not meet the NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods [1].

**Information Values:** Information mass fraction values for trace elements, glycerides, and ethanol are provided in Table 7. An information value is considered to be a value that will be of interest to the SRM user, but insufficient information is available to assess the uncertainty associated with the value or only a limited number of analyses were performed [1]. Information Values cannot be used to establish metrological traceability.

**Expiration of Certification:** The certification of **SRM 2772** is valid, within the measurement uncertainty specified, until **28 February 2019**, provided the SRM is handled and stored in accordance with the 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 technical measurements leading to certification were performed by J.A. Murray, M.M. Schantz, S.A. Wise, and W.E. May of the NIST Material Measurement Laboratory.

The development of SRM 2772 was a collaboration between NIST and INMETRO.

Statistical consultation for this SRM was provided by S.D. Leigh and J.H. Yen of the NIST Statistical Engineering Division.

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Analytical measurements at NIST were performed by B.E. Lang, J.A. Murray, L. Samuel (guest researcher from Measurements Standards Laboratory of New Zealand), M.M. Schantz, and L.J. Wood of the NIST Chemical Sciences Division; and T. Fortin and A. Laesecke of the NIST Applied Chemicals and Materials Division. Analytical measurements at INMETRO were performed by M.A. Gonçalves, S.P. Sobral, J.J.P. dos Santos, Jr., C.R.C. Rodrigues, F.B. Gonzaga, J.M. Rodrigues, G.F. da Cruz, and L.V.F. Gonçalves, and the measurements were coordinated by V.S. Cunha and R.J. Daroda. Cannon Instrument Company provided data for the kinematic viscosity at 40 °C.

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

## INSTRUCTIONS FOR HANDLING, STORAGE, AND USE

**Handling:** This material should be handled with care. Use proper disposal methods.

**Storage:** Sealed ampoules, as received, should be stored in the dark at temperatures lower than 30 °C.

**Use:** Sample aliquots for analysis (minimum sample size of 0.5 mL) should be withdrawn at 20 °C to 25 °C **immediately** after opening the ampoules and should be processed without delay for the certified values to be valid within the stated uncertainties. Transfer of material from the ampoule should be performed with maximum care so as not to contaminate the SRM.

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

**Source of Material:** The soy-based biodiesel for SRM 2772 was obtained from a farmer-owned cooperative, Ag Processing Inc. (AGP, Omaha, NE), and originated from their Methyl Ester Plant in Sergeant Bluff, Iowa (brand name – SoyGold).

**Preparation of Material:** The 55-gallon drum of material remained sealed until the material was ampouled. A 10 mL aliquot of the soy-based biodiesel was dispensed into each 10 mL amber ampoule that had been evacuated with argon and was then flame-sealed.

### Analytical Methods Used at NIST:

**Fatty Acid Methyl Esters:** Fatty acid methyl esters were measured by using combinations of two gas chromatographic (GC) methods with flame ionization detection (FID) or mass spectrometric (MS) detection. Two aliquots (0.5 mL, exact mass known) from each of twelve ampoules of SRM 2772 were chosen using a stratified random sampling scheme. These test portions were combined with approximately 25 mL isooctane in 30 mL amber bottles with Teflon-lined caps. To each bottle, 1 mL of the internal standard solution, consisting of tridecanoic acid methyl ester and nonadecanoic acid methyl ester in chloroform, was gravimetrically added using a gas-tight syringe. GC-FID analysis was performed using a 0.25 mm × 100 m SP2560 (nonbonded; biscyanopropyl polysiloxane) fused silica capillary column (Supelco, Bellefonte, PA), 0.25 µm film thickness. GC/MS was performed using a 0.25 mm × 60 m DB-23 (50 % cyanopropyl + 50 % phenylpolysiloxane, mole fraction) fused silica capillary column (Agilent Technologies, Wilmington, DE), 0.25 µm film thickness. The MS was operated in the scan mode (from 70 u to 400 u). Average response factors relative to the internal standards were calculated from independently prepared calibration solutions.

**Water:** Water was determined using a volumetric Karl Fischer technique, using anhydrous methanol as the solvent and titrated with Hydranal Composite 2 reagent. The ampoules of SRM were opened and immediately 2 mL to 3 mL of the biodiesel was aliquoted into dry 6 mL glass headspace vials. The sample vials were inserted into the Karl Fischer oven that was maintained at 140 °C to release the moisture from the samples. The resulting water vapor was transferred into the Karl Fischer titration cell using dry nitrogen as the carrier gas, flowing at a rate of 40 mL/min. Titrations of the water saturated octanol standard and the SRM were allowed to run for 30 min and 50 min, respectively, to fully measure the evolved moisture in the SRM.

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<sup>(1)</sup>Certain commercial equipment, instruments, or materials are identified in this report 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.

**Density, Viscosity, and Speed of Sound:** A density and sound speed analyzer was used to measure these two properties in the temperature range from 70 °C to 5 °C, and a viscodensimeter was used for the viscosity measurements and additional density measurements in the range from 100 °C to 10 °C.

**Methanol and Ethanol:** For analysis, 2.5 g (exact mass known) of the calibrant or biodiesel sample was mixed with 2.5 g of the internal standard solution, containing methanol-*d*<sub>4</sub> in water, in a 10 mL amber vial sealed with an open cap and Teflon-lined septum. The samples were heated at 70 °C for 5 min. A 5 min headspace solid-phase microextraction (SPME) sampling was performed by exposing the 100 µm polydimethylsiloxane fiber near the surface of the solution. GC/MS analysis was performed using a 0.32 mm × 15 m HP-Plot Q fused silica capillary column (Agilent Technologies), 20 µm film thickness. The SPME fiber was exposed in the injection port (220 °C) for 1 min. The split valve was closed for this 1 min and then opened to 20 mL/min. Ions 31 u, 35 u, and 45 u were monitored. Average response factors relative to the internal standards were calculated from independently prepared calibration solutions.

**Glycerides:** The derivatization procedure used for the glycerides in SRM 2772 was similar to that reported by Plank and Lorbeer [2]. One aliquot (0.2 g, exact mass known) was taken from each of six ampoules of SRM 2772 that were chosen using a stratified random sampling scheme. These test portions were combined with approximately 1 mL pyridine in 22 mL amber vials with Teflon-lined caps. To each vial, 0.25 mL of the internal standard solution, consisting of butanetriol and tricaprins in pyridine, was gravimetrically added using a gas-tight syringe. N-methyl-N-(trimethylsilyl)trifluoroacetamide (250 µL) was then added to each vial followed by vortexing. The solutions were allowed to sit at room temperature for 30 min and then 10 mL of heptane was added prior to GC-FID analysis. GC-FID analysis was performed using a 0.32 mm ID × 10 m MXT-Biodiesel TG capillary column with a 2 m × 0.53 mm ID guard column (Restek Corporation, Bellefonte, PA). All injections were performed on-column (1 µL) with helium as a carrier gas. Average response factors relative to the internal standards were calculated from independently prepared calibration solutions.

**Trace Elements:** Trace elements were determined using inductively coupled plasma optical emission spectrometry (ICP-OES). Aliquots of 2 g were taken from each of the six ampoules and placed in tall quartz beakers with HNO<sub>3</sub>. Indium was added to each sample solution as an internal standard to improve the precision of the instrumental measurements. The samples were heated on a hot plate with surface temperature of 100 °C and swirled continuously until an exothermic reaction occurred. At this point 4 mL of HClO<sub>4</sub> was added, and the samples were covered and heated overnight on a hot plate with surface temperature of 150 °C. The temperature was increased to 210 °C. Frequent additions of HNO<sub>3</sub> were needed to complete the digestion. Samples were eventually reduced to near dryness. Approximately 10 mL of 1.5 % HNO<sub>3</sub> (volume fraction) was added to each beaker, and the solutions were heated until clear. After complete digestion, the samples were removed from the hot plate, cooled, and then transferred to polyethylene bottles. The solutions were diluted to 25 g with 1.5 % HNO<sub>3</sub>. To determine calcium, iron, magnesium, and sodium, two 10 g aliquots were taken from the original solutions and transferred to weighed 30 mL polyethylene bottles. A spike containing calcium, iron, magnesium, and sodium was added to one of the aliquots for each sample. Analyte concentrations were calculated by the method of standard additions to compensate for any matrix effects. To determine copper, phosphorous, and potassium, a calibration curve was used to calibrate the instrument.

#### **Analytical Methods Used at INMETRO:**

**Fatty Acid Methyl Esters:** Fatty acid methyl ester concentrations were determined by GC/MS using a 0.32 mm × 30 m, 0.25 µm film thickness, CP WAX 52 CB fused silica capillary column (chemically bonded polyethylene glycol, Varian, Walnut Creek, CA). The MS was operated in the scan mode (from 50 u to 400 u). This GC/MS method permits the determination of fatty acid methyl esters with carbon chains from C8 to C24. An aliquot of 250 mg of ampouled soy-based biodiesel was dissolved in 5 mL of *n*-heptane for analysis.

**Water:** Water was determined using a coulometric Karl Fischer technique with Hydranal Coulomat AG as the reagent. The ampoules of soy-based biodiesel were opened and 2 g were aliquoted into a glass tube which was sealed immediately for analysis. The sealed vials were inserted into a Karl Fischer oven maintained at 170 °C. Dry nitrogen at 150 mL/min was used as the carrier gas. The time for water extraction was fixed at 300 s with measurements every 2 s.

**Glycerides:** The glycerides were determined using GC-FID in accordance with ASTM D6584-07 [3]. The results were used for confirmation of the assigned values.

**Trace Elements:** Sodium and potassium were determined using flame atomic absorption spectroscopy, and calcium, magnesium, and phosphorous were determined using ICP-OES.

**Density:** Density was determined using methods ABNT NBR 14065 [4] and ASTM D4052 [3].

**Kinematic Viscosity:** Kinematic viscosity was determined using methods ABNT NBR 10441 [4] and ASTM D446 (ISO 3105) [3].

**Methods Used at Cannon Instrument Company for Viscosity:** ASTM D445 [3] was used for the viscosity measurements on two instruments, a Cannon-Ubbelohde glass capillary viscometer and a Cannon miniAV-X automated viscometer.

**Homogeneity Assessment:** The homogeneity of the fatty acid methyl esters was assessed at NIST by using GC-FID. An analysis of variance did not indicate inhomogeneity for a 0.5 g sample. Other analytes were treated as though they were homogeneously distributed in the material although homogeneity was not assessed.

**Metrological Traceability for Certified Values:** The measurands are listed in Tables 1 to 3. Metrological traceability is to the SI derived units for mass fraction, density and kinematic viscosity; and are expressed as grams per kilogram; grams per 100 grams; grams per cubic centimeter and square millimeters per second, respectively.

Table 1. Certified Mass Fraction Values for Fatty Acid Methyl Esters in SRM 2772

	Mass Fraction (g/kg)
Tetradecanoic Acid Methyl Ester (C14:0) (Myristic Acid Methyl Ester)	0.755 ± 0.089 <sup>(a)</sup>
Octadecanoic Acid Methyl Ester (C18:0) (Stearic Acid Methyl Ester)	43.0 ± 2.7 <sup>(b)</sup>
(Z)-11-Octadecenoic Acid Methyl Ester (C18:1 n-7) (Vaccenic Acid Methyl Ester)	14.3 ± 1.5 <sup>(b)</sup>
Eicosanoic Acid Methyl Ester (C20:0) (Arachidic Acid Methyl Ester)	3.66 ± 0.52 <sup>(a)</sup>

<sup>(a)</sup> Certified values are unweighted means of the results from three to four analytical methods at NIST with confirmatory data from INMETRO. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor  $k = 2$ , calculated by combining a between-method variance [5] with a pooled, within-method variance following the ISO/JCGM Guide [6].

<sup>(b)</sup> Certified values are unweighted means of the results from three to four analytical methods at NIST with confirmatory data from INMETRO. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor  $k = 3$ , calculated by combining a between-method variance [5] with a pooled, within-method variance following the ISO/JCGM Guide [6].

Table 2. Certified Mass Fraction Value for Water in SRM 2772

	Mass Fraction <sup>(a)</sup> (%)
Water	0.018 ± 0.002

<sup>(a)</sup> The certified value is the unweighted mean of the results from two analytical methods at NIST and INMETRO. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor  $k = 2$ , calculated by combining a between-method variance [5] with a pooled, within-method variance following the ISO/JCGM Guide [6].

Table 3. Certified Value for Density at 20 °C and Kinematic Viscosity at 20 °C, 30 °C, and 40 °C of SRM 2772

	Value		
Density at 20 °C <sup>(a)</sup>	0.88132	± 0.00006	g/cm <sup>3</sup>
Kinematic Viscosity at 20 °C <sup>(b)</sup>	6.4310	± 0.0098	mm <sup>2</sup> /s
Kinematic Viscosity at 30 °C <sup>(b)</sup>	5.0532	± 0.0069	mm <sup>2</sup> /s
Kinematic Viscosity at 40 °C <sup>(c)</sup>	4.0843	± 0.0057	mm <sup>2</sup> /s

<sup>(a)</sup> The certified value is the unweighted mean of the results from three analytical methods, two at NIST and one at INMETRO. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor  $k = 2$ , calculated by combining a between-method variance [5] with a pooled, within-method variance following the ISO/JCGM Guide [6].

<sup>(b)</sup> The certified value is the unweighted mean of the results from two analytical methods at NIST and INMETRO. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor  $k = 2$ , calculated by combining a between-method variance [5] with a pooled, within-method variance following the ISO/JCGM Guide [6].

<sup>(c)</sup> The certified value is the unweighted mean of the results from three analytical methods at NIST, INMETRO, and Cannon Instrument Company. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor  $k = 2$ , calculated by combining a between-method variance [2] with a pooled, within-method variance following the ISO/JCGM Guide [3].

**Metrological Traceability for Reference Values:** Based on the methods used, the measurands are listed in Tables 4 to 6. Metrological traceability is to the SI derived units for mass fraction, density, kinematic viscosity and velocity, and are expressed as grams per kilogram; milligrams per kilogram; grams per cubic centimeter; square millimeters per second and meters per second; respectively.

Table 4. Reference Mass Fraction Value for Docosanoic Acid Methyl Ester in SRM 2772

	Mass Fraction <sup>(a)</sup> (g/kg)		
Docosanoic Acid Methyl Ester (C22:0) (Behenic Acid Methyl Ester)	3.7	± 1.1	

<sup>(a)</sup> The reference value is unweighted means of the results from three analytical methods at NIST with confirmatory data from INMETRO. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor  $k = 2$ , calculated by combining a between-method variance [5] with a pooled, within-method variance following the ISO/JCGM Guide [6].

Table 5. Reference Mass Fraction Value for Methanol in SRM 2772

	Mass Fraction <sup>(a)</sup> (mg/kg)		<i>t</i> -value
Methanol	587	± 44	2.36

<sup>(a)</sup> The reference value is the means of the results obtained using one analytical technique at NIST. The expanded uncertainty,  $U$ , is calculated as  $U = k u_c$ , where  $u_c$  is one standard deviation of the mean, and the coverage factor,  $k$ , is determined from the Student's  $t$ -distribution corresponding to the associated degrees of freedom and a 95 % confidence level for each analyte.

Table 6. Reference Values<sup>(a,b)</sup> as a Function of Temperature for Density, Kinematic Viscosity, and Speed of Sound of SRM 2772

Temperature (°C)	Density (g/cm <sup>3</sup> )	Kinematic Viscosity (mm <sup>2</sup> /s)	Speed of Sound (m/s)
5	0.8923 ± 0.0001		1467.7 ± 0.1
10	0.8886 ± 0.0001	8.494 ± 0.022	1449.1 ± 0.1
15	0.8850 ± 0.0001	7.359 ± 0.018	1430.7 ± 0.1
20 <sup>(c)</sup>			1412.5 ± 0.1
25	0.8776 ± 0.0001	5.683 ± 0.011	1394.5 ± 0.1
30 <sup>(c)</sup>	0.8740 ± 0.0001		1376.7 ± 0.2
35	0.8704 ± 0.0001	4.533 ± 0.008	1359.1 ± 0.2
40 <sup>(c)</sup>	0.8667 ± 0.0001		1341.6 ± 0.2
45	0.8630 ± 0.0001	3.712 ± 0.005	1324.3 ± 0.3
50	0.8594 ± 0.0001	3.388 ± 0.004	1307.1 ± 0.3
55	0.8558 ± 0.0001	3.107 ± 0.004	1290.1 ± 0.3
60	0.8521 ± 0.0001	2.862 ± 0.003	1273.2 ± 0.3
65	0.8485 ± 0.0001	2.647 ± 0.003	1256.6 ± 0.4
70	0.8449 ± 0.0001	2.457 ± 0.002	1240.1 ± 0.4
75	0.8412 ± 0.0001	2.289 ± 0.002	
80	0.8376 ± 0.0001	2.138 ± 0.001	
85	0.8340 ± 0.0001	2.002 ± 0.001	
90	0.8303 ± 0.0001	1.880 ± 0.001	
95	0.8267 ± 0.0001	1.769 ± 0.001	
100	0.8231 ± 0.0001	1.667 ± 0.001	

<sup>(a)</sup> The reference values are the means of results using one analytical technique. The expanded uncertainty,  $U$ , is calculated as  $U = k u_c$ , where  $u_c$  is one standard deviation of the analyte mean, and the coverage factor,  $k$ , is determined from the Student's  $t$ -distribution corresponding to the associated degrees of freedom and a 95 % confidence level for each analyte ( $t$ -value = 2.78).

<sup>(b)</sup> Based on measurements at NIST

<sup>(c)</sup> See Table 3

Table 7. Information Mass Fraction Values for Trace Elements, Glycerides, and Ethanol in SRM 2772

	Mass Fraction (mg/kg)
Calcium <sup>(a)</sup>	0.5
Copper <sup>(b)</sup>	<0.2 <sup>(c)</sup>
Diglycerides <sup>(b)</sup>	1960
Ethanol <sup>(b)</sup>	<0.2 <sup>(c)</sup>
Iron <sup>(b)</sup>	<0.2 <sup>(c)</sup>
Magnesium <sup>(a,b)</sup>	<0.2 <sup>(c)</sup>
Monoglycerides <sup>(b)</sup>	3620
Phosphorus <sup>(a,b)</sup>	<0.4 <sup>(c)</sup>
Potassium <sup>(a,b)</sup>	<0.1 <sup>(c)</sup>
Sodium <sup>(a)</sup>	0.07
Total Glycerin <sup>(b)</sup>	1520
Triglycerides <sup>(b)</sup>	1230

<sup>(a)</sup> Based on measurements at INMETRO

<sup>(b)</sup> Based on measurements at NIST

<sup>(c)</sup> The method detection limits were estimated by taking three times the standard deviation of the ten or more blanks that were run along with the samples.

#### REFERENCES

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- [2] Plank, C.; Lorbeer, E.; *Simultaneous Determination of Glycerol, and Mono-, Di-, and Triglycerides in Vegetable Oil Methyl Esters by Capillary Gas Chromatography*; J. Chromatogr. A, Vol. 697, pp. 461–468 (1995).
- [3] ASTM D6584 Standard Test Method for Determination of Free and Total Glycerin in B-100 Biodiesel Methyl Esters By Gas Chromatography; ASTM D4052 Standard Test Method for Density and Relative Density of Liquids by Digital Density Meter; ASTM D446 Standard Specifications and Operating Instructions for Glass Capillary Kinematic Viscometers; ASTM D445 Standard Test Method for Kinematic Viscosity of Transparent and Opaque Liquids (and Calculation of Dynamic Viscosity), available at <http://www.astm.org> (accessed Oct 2016).
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- [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 GUM*; J. Res. Natl. Inst. Stand. Technol., Vol. 105, pp. 571–579 (2000).
- [6] 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 (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 Oct 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 Oct 2016).

**Certificate Revision History:** **20 October 2016** (Change of expiration date; removal of selected certified and reference values for fatty acid methyl esters (Tables I and 4) and reference values for physical and chemical properties (Table 5) due to instability; editorial changes); **20 December 2012** (Certified mass fraction value for  $\alpha$ -linolenic acid was revised to a reference value); **28 September 2011** (Addition of  $t$ -values; editorial changes); **04 December 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>.*