National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 3253

Yerba Mate Leaves

This Standard Reference Material (SRM) is intended primarily for use in validating methods for determining polycyclic aromatic hydrocarbons (PAHs) in yerba mate and similar matrices, and can also be used for quality assurance when assigning values to in-house control materials. A unit of SRM 3253 consists of two heat-sealed, aluminized pouches, each containing approximately 10 g of ground yerba mate.

The development of SRM 3253 was a collaboration between the National Institute of Standards and Technology (NIST) and the National Institutes of Health (NIH) Office of Dietary Supplements (ODS).

Certified Mass Fraction Values: Certified mass fraction values for selected PAHs, reported on a dry-mass basis, 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; certified values were calculated as the unweighted means of the mean values from NIST methods. The associated uncertainties are expressed at an approximately 95 % level of confidence [2–4].

Reference Mass Fraction Values: Reference mass fraction values for additional PAHs and proximates, reported on a dry-mass basis, are provided in Tables 2 and 3. 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. The reference mass fraction values for PAHs were calculated from results reported by NIST, and values for proximates are based on data from a collaborating laboratory.

Expiration of Certification: The certification of **SRM 3253** is valid, within the measurement uncertainty specified, until **01 January 2028**, 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 M.M. Phillips of the NIST Chemical Sciences Division and K.E. Sharpless of the NIST Special Programs Office.

Analyses at NIST were performed by K.D. Chieh, J.A. Murray, R.L. Paul, M.M. Schantz, and L.J. Wood of the NIST Chemical Sciences Division.

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

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Certificate Issue Date: 12 April 2018

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

NOTICE TO USERS: SRM 3253 IS INTENDED FOR LABORATORY USE ONLY, NOT FOR HUMAN CONSUMPTION.

INSTRUCTIONS FOR STORAGE AND USE

Storage: The SRM should be stored at controlled room temperature (20 °C to 25 °C) in the original unopened packets. The certification only applies to the initial use, as the material will take on additional moisture upon opening the packet. The same results are not guaranteed if the remaining powder is used at a later date unless an independent determination of moisture is conducted using one of the methods listed under "Determination of Moisture".

Use: Before use, the contents of the packet should be mixed thoroughly. Allow the contents to settle for 1 min prior to opening to minimize the loss of fine particles. To relate analytical determinations to the certified or reference values in this certificate, a test portion mass of at least 0.3 g should be used. Results obtained in analyses should include their own estimates of uncertainty and can be compared to the certified or reference values using procedures described in reference 6.

Determination of Moisture: Moisture content of SRM 3253 was determined at NIST by (1) freeze-drying to constant mass over 7 d, (2) drying over magnesium perchlorate in a desiccator at room temperature for 28 d; and (3) drying for 2 h in a forced-air oven at 90 °C. Unweighted results obtained using all three techniques were averaged to determine a conversion factor of (0.9566 ± 0.0023) gram dry-mass per gram as-received mass, which was used to convert data from an as-received to a dry-mass basis; the uncertainty shown on this value is an expanded uncertainty. A relative uncertainty component (0.12 %) for the conversion factor obtained from the moisture measurements is incorporated in the uncertainties of the certified and reference values, reported on a dry-mass basis, that are provided in this certificate.

SOURCE, PREPARATION, AND ANALYSIS[1]

Source and Preparation: SRM 3253 is a ground, homogenized, commercially available yerba mate. The commercial product was ground at NIST using an ultracentrifugation mill, and the fine powder was transferred to High-Purity Standards (Charleston, SC). The material was packaged in 10 g aliquots in heat-sealed 4 mil polyethylene bags then sealed inside nitrogen-flushed aluminized plastic bags along with two silica gel packets. Following packaging, SRM 3253 was irradiated (Neutron Products, Inc., Dickerson, MD) to an absorbed dose of 5.9 kGy to 7.6 kGy.

Analytical Approach for Determination of PAHs: Value assignment of the mass fractions of PAHs in SRM 3253 was based on the combination of measurements made at NIST using three methods based on gas chromatography with mass spectrometric detection (GC-MS).

NIST Analyses for PAHs by GC-MS Method I: For GC-MS Method I, mass fractions of PAHs were determined in two 0.3 g test portions from each of 10 packets of SRM 3253. The yerba mate and internal standard solution (prepared as a gravimetric dilution of SRM 2269 *Perdeuterated PAH-I Solution in Hexane/Toluene* and SRM 2270 *Perdeuterated PAH-II Solution in Hexane/Toluene* in 2,2,4-trimethylpentane) were mixed with sodium sulfate in glass extraction thimbles. Following Soxhlet extraction with hexane:acetone (1:1 volume fraction) for 20 h, the extract was evaporated to 5 mL under nitrogen and centrifuged. The supernatant was fractionated using an aminopropyl solid phase extraction (SPE) column to isolate the fraction of interest. The processed extract was evaporated to 0.5 mL under nitrogen and analyzed by GC-MS. The GC was equipped with a 0.25 mm i.d. × 60 m Rxi®-PAH fused silica capillary column (Restek, Bellefonte, PA) with a 0.1 μm film thickness. All injections were 1 μL (on-column) with helium as a carrier gas at a constant flow rate of 1.2 mL/min. Electron impact MS was used for detection. Calibrants were prepared gravimetrically from *SRM 2260a Aromatic Hydrocarbons in Toluene* and *SRM 1491a Methyl-Substituted Polycyclic Aromatic Hydrocarbons in Toluene* at levels intended to approximate the levels of the PAHs in SRM 3253 following extraction. The same internal standard solution was used for the calibrants and samples. Calculations are based on linear regression of response data for the calibrants.

NIST Analyses for PAHs by GC-MS Method II: For GC-MS method II, mass fractions of PAHs were determined from single 0.5 g test portions from each of six packets of SRM 3253. The yerba mate and internal standard solution

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[1] 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.

(prepared as a gravimetric dilution of *SRM 2269 Perdeuterated PAH-I Solution in Hexane/Toluene* and *SRM 2270 Perdeuterated PAH-II Solution in Hexane/Toluene* in 2,2,4-trimethylpentane) were mixed with diatomaceous earth in pressurized fluid extraction (PFE) cells. Following PFE with dichloromethane in duplicates of 6 cycles at 150 °C, the duplicate extracts were combined, evaporated to dryness under nitrogen, and reconstituted in 0.5 mL of hexane. The extract was fractionated using aminopropyl SPE columns to isolate the fraction of interest. The processed extract was evaporated to 0.5 mL under nitrogen and analyzed by GC-MS. The GC was equipped with a 0.25 mm i.d. × 59 m DB-XLB fused silica capillary column (Agilent Technologies, Wilmington, DE) with a 0.25 μm film thickness. All injections were 1 μL (splitless) with helium as a carrier gas at a constant flow rate of 1.2 mL/min. Electron impact MS was used for detection. Calibrants were prepared gravimetrically from *SRM 2260a Aromatic Hydrocarbons in Toluene* and *SRM 1491a Methyl-Substituted Polycyclic Aromatic Hydrocarbons in Toluene*, at levels intended to approximate the levels of the PAHs in SRM 3253 following extraction. The same internal standard solution was used for the calibrants and samples. Calculations are based on linear regression of response data for the calibrants.

NIST Analyses for PAHs by GC-MS Method III: For GC-MS Method III, mass fractions of PAHs were determined in three 0.5 g test portions from each of three packets of SRM 3253. The yerba mate was mixed with diatomaceous earth in PFE cells. An internal standard solution (prepared as a gravimetric dilution of SRM 2269 *Perdeuterated PAH-I Solution in Hexane/Toluene* and SRM 2270 *Perdeuterated PAH-II Solution in Hexane/Toluene* in 2,2,4-trimethylpentane). Following PFE with dichloromethane in duplicates of 6 cycles at 150 °C, the duplicate extracts were combined, solvent exchanged to hexane, and evaporated to a final volume of 0.5 mL. The extract was fractionated using silica SPE columns to isolate the fraction of interest. The processed extract was evaporated to 0.5 mL under nitrogen and analyzed by GC-MS. The GC was equipped with a 0.25 mm i.d. × 60 m Rxi®-PAH fused silica capillary column (Restek) with a 0.15 μm film thickness. All injections were 1 μL (splitless) with helium as a carrier gas at a constant flow rate of 1.2 mL/min. Electron impact MS was used for detection. Calibrants were prepared gravimetrically from *SRM 2260a Aromatic Hydrocarbons in Toluene*, at levels intended to approximate the levels of the PAHs in SRM 3253 following extraction. The same internal standard solution was used for the calibrants and samples. Calculations are based on the average response factor relative to the labeled internal standard of the calibrants bracketing samples and controls.

Collaborating Laboratory's Analyses: The collaborating laboratory used their usual methods to make single measurements of proximates and calories on test portions taken from each of three packets of SRM 3253. The laboratory mean was used for value assignment, with the uncertainty estimated as the standard error of that mean.

Homogeneity Assessment: The homogeneity of PAHs was assessed using GC-MS Method I. Analyses of variance with a 5 % significance level showed statistically significant heterogeneity for fluoranthene, pyrene, benzo[c]phenanthrene, triphenylene, chrysene, benzo[b]fluoranthene, and benzo[a]fluoranthene. The uncertainties for these PAHs incorporate an additional component for possible heterogeneity.

Value Assignment: For calculation of assigned values for PAHs, the mean of the mean values from GC-MS Method I, GC-MS Method II, and GC-MS method III results was used, where appropriate. Assigned values for proximates were based on the mean of values from a collaborating laboratory.

Certified Mass Fraction Values for PAHs: Each certified mass fraction value is the combined mean from the means of results from analyses provided by GC-MS Method I, GC-MS Method II, and GC-MS Method III, where appropriate. The measurand is the total mass fraction of each PAH listed in Table 1. Metrological traceability is to the SI derived unit for mass fraction (expressed as nanograms per gram) on a dry-mass basis.

Table 1. Certified Mass Fraction Values for PAHs in SRM 3253

	Mass Fraction[a] (ng/g)		
Pyrene[b,c,d]	324	±	53
Benz[a]anthracene[b,c,d]	70.2	±	3.0
Benzo[a]pyrene[b,c,d]	57.8	±	6.9
Perylene[b,d]	14.4	±	2.7
Benzo[ghi]perylene[b,c,d]	118.6	±	2.3

[a] 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 true value of the analyte is believed to lie within the interval $x \pm U_{95\%}(x)$ with about a 95 % confidence. To propagate this uncertainty, treat the value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$.
[b] GC-MS Method I
[c] GC-MS Method II
[d] GC-MS Method III

Reference Mass Fraction Values for PAHs: Each reference mass fraction value is the mean or combined mean from one or more of the three GC-MS method results. The measurand is the mass fraction of each PAH listed in Table 2. Metrological traceability is to the SI derived unit for mass fraction (expressed as nanograms per gram) on a dry-mass basis, as realized by the methods used.

Table 2. Reference Mass Fraction Values for PAHs in SRM 3253

	Mass Fraction[a] (ng/g)		
2-Methylphenanthrene[b,c]	69	±	10
3-Methylphenanthrene[b,c]	62	±	14
Fluoranthene[d]	280	±	48
1-Methylpyrene[b,c]	18.8	±	1.6
Benzo[c]phenanthrene[b,c,d]	18.9	±	2.4
Chrysene[d]	128	±	16
Triphenylene[d]	29.1	±	6.9
Benzo[b]fluoranthene[b,d]	48	±	22
Benzo[k]fluoranthene[c,d]	25.1	±	9.1
Benzo[a]fluoranthene[b,d]	14.7	±	3.8
Indeno[1,2,3-cd]pyrene[b,c]	57.7	±	4.2
Picene[d]	12.72	±	0.58
Coronene[b]	43.8	±	1.8

[a] 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 true value of the analyte is believed to lie within the interval $x \pm U_{95\%}(x)$ with about a 95 % confidence. To propagate this uncertainty, treat the value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$.
[b] GC-MS Method I
[c] GC-MS Method II
[d] GC-MS Method III

Reference Mass Fraction Values for Proximates and Calories: Each reference mass fraction value is the mean from collaborating laboratory results. The measurands are the mass fractions listed in Table 3, on a dry-mass basis, as determined by the methods indicated. Metrological traceability is to mass fraction (expressed as grams per 100 grams) as realized by the methods used. For calories, the measurand is the caloric content (expressed as kilocalories per 100 grams), listed in Table 3 on a dry-mass basis as determined by the method indicated and metrological traceability is to the scale realized by that method for energy.

Table 3. Reference Mass Fraction Values for Proximates and Calories in SRM 3253

	Mass Fraction[a] (g/100 g)	
Ash[b]	5.81 ±	0.17
Protein[c]	10.64 ±	0.90
Carbohydrates[d]	77.8 ±	3.1
Total Fat[e]	5.4 ±	3.4

	Mass Fraction[a] (kcal/100 g)	
Calories	402 ±	15

[a] 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 true value of the analyte is believed to lie within the interval $x \pm U_{95\%}(x)$ with about a 95 % confidence. To propagate this uncertainty, treat the value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$.

[b] Ash was determined by a collaborating laboratory using weight loss after ignition in muffle furnace.

[c] Nitrogen was determined by a collaborating laboratory using combustion. A factor of 6.25 was used to convert nitrogen results to protein.

[d] Carbohydrates were determined by a collaborating laboratory by difference (solids less the sum of protein, fat, and ash).

[e] Total fat was determined by a collaborating laboratory using the Mojonnier method.

REFERENCES

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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; or via the Internet at https://www.nist.gov/srm.