National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 3250

Saw Palmetto (*Serenoa repens*) Fruit

This Standard Reference Material (SRM) is intended primarily for use in evaluating analytical methods for the determination of phytosterols in the fruit of *Serenoa repens* (saw palmetto) and similar matrices. This SRM can also be used for quality assurance when assigning values to in-house control materials. This SRM has also been characterized for its DNA sequence. A unit of SRM 3250 consists of five packets, each containing approximately 6 g of ground saw palmetto fruit.

The development of SRM 3250 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). The addition of genetic information was accomplished through collaboration among NIST, NIH ODS, the U.S. Department of Agriculture (USDA) Agricultural Research Service (ARS), NSF International (formerly AuthenTechnologies®, Richmond, CA), and American Herbal Pharmacopoeia (Scotts Valley, CA).

Taxonomic Identification: The taxonomic identity is *Serenoa repens*, established through identification by a trained botanist using an herbarium specimen from original material and from associated DNA sequence analysis from botanically authenticated *Serenoa repens.* The associated DNA sequences are available in companion FASTA-formatted files [1]. The uncertainty associated with each nucleotide in the sequence, and in turn the uncertainty associated with the DNA sequence as an identifier of species, is expressed in an ordinal scale that represents the confidence estimates of the assigned value (Tables 1 and 2) [2]. These DNA sequences are used as a source of identity data for *Serenoa repens.*

Certified 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 [3]. The certified mass fraction values of selected phytosterols are provided in Table 3 on a dry-mass basis. Values were derived from the combination of results provided by NIST using two independent methods. The certified values in this material are the equally weighted means of the results of the individual sets of data; the associated uncertainties are expanded uncertainties at the 95 % level of confidence [4–6].

Expiration of Certification: The certification of **SRM 3250** is valid, within the measurement uncertainty specified, until **30 November 2029**, provided the SRM is handled and stored in accordance with the 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 C.A. Rimmer, L.C. Sander, and S.A. Wise of the NIST Chemical Sciences Division and K.E. Sharpless of the NIST Office of Special Programs.

Carlos A. Gonzalez, Chief
Chemical Sciences Division

Gaithersburg, MD 20899
Certificate Issue Date: 27 January 2021
Certificate Revision History on Page 6

Steven J. Choquette, Director
Office of Reference Materials

Analytical measurements at NIST were performed by M. Bedner and L.J. Wood of the NIST Chemical Sciences Division, and B.J. Porter, and M.M. Schantz formerly of NIST.

Support for the development of SRM 3250 was provided in part by NIH ODS. Acquisition of the material was coordinated by A. Nguyenpho of FDA CDER and K.E. Sharpless of the NIST Office of Special Programs.

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

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

NOTICE AND WARNING TO USERS: SRM 3250 IS INTENDED FOR RESEARCH USE; NOT FOR HUMAN CONSUMPTION.

INSTRUCTIONS FOR STORAGE AND USE

Storage: The material should be stored at controlled room temperature (20 °C to 25 °C), in an unopened packet, until required for use.

Use: Prior to removal of a test portion for analysis, the contents of a packet of material should be mixed thoroughly. For certified values to be valid, test portions of the powder equal to or greater than 0.5 g for phytosterol analysis should be used. When the contents of an opened packet have been transferred to a clean amber screw-cap bottle and stored in a desiccator, phytosterols have been found to be stable for 5 days. Test portions should be analyzed as-received and results converted to a dry-mass basis by determining moisture content (described below) on a separate test portion.

Determination of Moisture: Moisture content of SRM 3250 was determined at NIST by (1) freeze-drying to constant mass over 7 days; (2) drying over magnesium perchlorate in a desiccator at room temperature for 38 d; and (3) drying for 4 h in a forced-air oven at 100 °C. The results from all three techniques were averaged to determine a dry-mass proportion of (0.9358 ± 0.0097) gram dry-mass per gram as-received mass; the uncertainty shown on this value is an expanded uncertainty. The conversion factor used to convert data from as-received to a dry-mass basis is the inverse of the dry-mass proportion. A relative uncertainty component for the conversion factor (0.24 %) obtained from the moisture measurements is incorporated in the uncertainties of the certified values, reported on a dry-mass basis, that are provided in this certificate.

PREPARATION AND ANALYSIS[(1)]

Material Acquisition and Preparation: The material (ground fruit) for production of SRM 3250 was packaged as-received. The ground fruit of *Serenoa repens* was heat-sealed inside a nitrogen-flushed 4 mil polyethylene bag, which was then sealed inside a nitrogen-flushed aluminized plastic bag along with two packets of silica gel. Following packaging, SRM 3250 was irradiated (Neutron Products, Inc.; Dickerson, MD) at an absorbed dose of 3.6 kGy to 4.5 kGy.

NIST ANALYSES FOR PHYTOSTEROLS

Value assignment of the mass fractions of the phytosterols in SRM 3250 was based on the combination of results from two different analytical methods at NIST. Phytosterols were measured at NIST using GC with FID and LC with MS detection. Four independently prepared calibrants were used for each of the methods. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the phytosterols in the SRM. A single internal standard solution was used for the calibrants and samples.

Sample Preparation Method 1: Single 3 g test portions from each of eight packets were individually combined with an internal standard solution containing cholesterol and were saponified using an ethanolic potassium hydroxide (KOH) solution. Phytosterols were extracted into toluene, which was then evaporated under nitrogen. Phytosterols were derivatized using hexamethyldisilane and trimethylchlorosilane and analyzed by using GC-FID.

Sample Preparation Method 2: Two 0.5 g test portions from each of six packets were individually combined with an internal standard solution containing cholesterol, ethanol, and hydrochloric acid. After heating for 30 min, KOH was added, and the solution was refluxed for 45 min. Analytes were then extracted into toluene, which was then

[(1)] Certain commercial equipment, instruments, or materials are identified in this Certificate 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.

evaporated under nitrogen, and the residue reconstituted in isopropanol. Extracts were filtered and analyzed by using LC-MS.

GC with Flame Ionization Detection: GC-FID was performed using a 0.25 mm × 30 m fused silica capillary column containing a dimethylpolysiloxane phase. Peak identities were confirmed by using a GC x GC time-of-flight mass spectrometry system. A typical chromatogram is provided in Appendix A.

LC with Mass Spectrometric Detection: LC with atmospheric pressure chemical ionization MS was performed using a 15 cm C18 column. The following ions (*m/z*) were monitored: 369 (cholesterol, internal standard), 383 (campesterol), 395 (stigmasterol), and 397 (β-sitosterol). Campesterol and stigmasterol coeluted in the chromatogram, and the peak area for stigmasterol at *m/z* 395 was corrected for a small contribution from campesterol based on the calibrant responses. A typical chromatogram is provided in Appendix A.

ASSIGNMENT OF IDENTITY

Molecular Approach for Species Identity: Sanger sequencing was used on two independent chloroplast gene regions, the *psbA-trnH* intergenic spacer region and the *trnL-trnF* intergenic spacer region [7–9] for authentication of SRM 3250. Complete sequencing of the strands of DNA for the two gene regions was performed on triplicate samples, the sequence reads were independently hand-curated, and the confidence values were estimated as described below. DNA sequences are available in companion FASTA-formatted files [1]. To certify the species identity, validation studies were performed based on the "Probability of Identification (POI)" model using an Inclusivity and Exclusivity Panel [10]. Multiple samples of authenticated herbarium vouchers, botanical identity reference materials, and published literature were used to comprise the panels from the target and most closely related species (see Appendix B for more information on the reference samples). These samples were analyzed a minimum of ten times to ensure consistency in methods from replicate to replicate. Maximum Likelihood (ML) phylogenetic analyses were performed using the phylogenetic estimation using ML (PhyML) algorithm, with a General Time Reversible (GTR) substitution model, a fixed transition-transversion ratio, and 1,000 bootstrap replicates. ML phylogenies, and the DNA aligned matrices, which can be found in Appendices C and D, were used to determine the species identity of SRM 3250 and to estimate the confidence (as described below). The confidence level for the *psbA-trnH* and *trnL-trnF* intergenic regions was "Most Confident" (0).

The taxonomic identification is *Serenoa repens* and the associated chloroplast DNA sequences from the *psbA-trnH* intergenic spacer region and the *trnL-trnF* intergenic spacer region are available in companion FASTA-formatted files [1]. The confidence associated with each nucleotide in the sequence, and in turn the confidence associated with the DNA sequence as an identifier of species, is expressed in an ordinal scale that represents the strength of the belief in the assigned value (Table 1) [2]. In the absence of a fully developed metrology for identity, these DNA sequences are used as a source of identity data for *Serenoa repens.* Chloroplast and nuclear ribosomal DNA sequences from botanically authenticated *Serenoa repens* samples are used to establish inclusivity; chloroplast and nuclear ribosomal DNA sequences from close relatives are used to establish exclusivity.

Nucleotide Identity: A set of heuristic, experience-based, rules (see Table 2) were used to establish confidence estimates for the nucleotides comprising the DNA sequences obtained from SRM 3250, and their use in identifying the species (see Table 1). The DNA sequences with curated confidence estimates are available in companion FASTA-formatted files [1]. The DNA aligned matrices used to determine the species identity and their confidence estimates are provided in Appendices C and D. The confidence associated with each nucleotide in the sequence in the FASTA files provided [1], and in turn the confidence associated with the DNA sequence as an identifier of species, is expressed in an ordinal scale that represents the strength of the belief in the assigned value (Table 2) [2]. Characteristics of sequence data and phylogenetic data used for species identity associated with the levels of the ordinal scale are described in Tables 1 and 2. The confidence estimates for the *psbA-trnH* and *trnL-trnF* sequences are available in companion FASTA-formatted files [1].

Table 1. Definitions of Heuristic Rules for Confidence Estimates of Species Identity

Confidence Level	Species Identity
Most Confident (0)	Have very well-supported and well-resolved phylogeny and/or multiple diagnostic nucleotides differentiating species from closest relatives; have data from multiple samples of both an inclusivity and exclusivity panel; data from multiple independent gene regions agree.
Very Confident (1)	Have reasonably well-supported and well-resolved phylogeny and/or a few diagnostic nucleotides differentiating species from close relatives; have data from multiple samples of both an inclusivity and exclusivity panel; data from one gene, or data from multiple independent gene regions agree.
Confident (2)	Have reasonably well-supported and well-resolved phylogeny and/or one or a few diagnostic nucleotides differentiating species from close relatives; have data from a few samples of both an inclusivity and exclusivity panel; data from one gene, or data from multiple independent gene regions generally agree.
Ambiguous (3)	Have a poorly supported and poorly resolved phylogeny and/or no diagnostic nucleotides differentiating species from close relatives; have data from a few or multiple samples of both an inclusivity and exclusivity panel; data from one gene, or data from multiple independent gene regions generally disagree.

Table 2. Definitions of Heuristic Rules for Confidence Estimates of DNA Nucleotide Identity

Confidence Level	Nucleotide Identity
Most Confident (0)	Have good answers (fully reliable, unambiguous base calls) on both strands; all data from both strands agree.
Very Confident (1)	Have good answer on one strand; poor answer (less than fully reliable, potentially ambiguous base call) on the second/alternate strand; base calls from both strands typically agree, and there is biochemical context that explains the anomalous sequence data.
Confident (2)	Have good answer on one strand; anomalous sequence data that may give rise to a conflicting base call on the alternate strand; judgment required to resolve anomaly.
Ambiguous (3)	No clear mutually supporting results; unambiguous base calls disagree; or no unambiguous base calls on either strand; data from the two opposing strands could not be authoritatively reconciled.

Homogeneity Assessment: The homogeneity of phytosterols was assessed at NIST by using the LC-MS method described above. An analysis of variance did not show inhomogeneity for the test portions analyzed (0.5 g).

Value Assignment: The equally weighted means from each set of data available were used to calculate the assigned values.

Table 3. Certified Mass Fraction Values for Selected Phytosterols in SRM 3250[(a)]

Phytosterols	Mass Fraction (mg/g)		
Campesterol	0.1175	±	0.0025
β-Sitosterol	0.454	±	0.018
Stigmasterol	0.0477	±	0.0020

(a) Each certified mass fraction value is an equally weighted mean of results provided by GC-FID and LC/MS. Values are expressed as $x \pm U_{95\%}(x)$, where x is the certified value and $U_{95\%}(x)$ is the expanded uncertainty of the certified value. The true value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with 95 % confidence. To propagate this uncertainty, the certified value should be treated as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [3,4]. The measurands are the mass fractions of the selected phytosterols listed in Table 3, on a dry-mass basis. Metrological traceability is to the measurement unit as realized through the purity determined for the primary chemical standards employed in the NIST methods.

REFERENCES

[1] Certified data can be downloaded from the "Data and Information Files" link for the SRM available at https://www-s.nist.gov/srmors/view_detail.cfm?srm=3250.
— The certified confidence estimates for every base of *psbA-trnH* intergenic region sequence data file is: CF Serenoa_SRM3250_pbsA_Nucleotide_Identity_v1.docx
— The certified sequence data file of *psbA-trnH* intergenic region sequence is: SRM3250_pbsA_sequence_v1.docx
— The certified confidence estimates for every base of *trnL-trnF* intergenic region sequence file is: CF Serenoa_SRM3250_trnL_Nucleotide_Identity_v1.docx
— The certified sequence data file of *trnL-trnF* intergenic region sequence is: SRM3250_trnL_sequence_v1.docx

[2] SRM 2374; *DNA Sequence Library for External RNA Controls*; National Institute of Standards and Technology; U.S. Department of Commerce: Gaithersburg, MD (20 March 2013); available at https://www-s.nist.gov/srmors/view_detail.cfm?srm=2374 (accessed Jan 2021).

[3] 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.; Phinney, K.W.; Polakoski, M.; Possolo, A.; Sharpless, K.E.; Sieber, J.R.; 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 260-136; U.S. Government Printing Office: Washington, DC (2020); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2020.pdf (accessed Jan 2021).

[4] 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 https://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Jan 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 Jan 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).

[6] 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 Jan 2021).

[7] Sang, T.; Crawford, D.J.; Stuessy, T.F.; *Chloroplast DNA Phylogeny, Reticulate Evolution, and Biogeography of Paeonia (Paeoniaceae)*; Am. J. Bot., Vol. 84, pp. 1120–1136 (1997).

[8] Tate, J.A.; Simpson, B.B.; *Paraphyly of Tarasa (Malvaceae) and Diverse Origins of the Polyploid Species*; Syst. Bot., Vol. 28, pp. 723–737 (2003).

[9] Taberlet, P.; Gielly, L.; Pautou, G.; Bouvet, J.; *Universal Primers for Amplification of Three Non-coding Regions of Chloroplast DNA*; Plant Mol. Bio., Vol. 17, pp. 1105–1109 (1991).

[10] Labudde, R.A.; Harnly, J.M.; *Probability of identification: A Statistical Model for the Validation of Qualitative Botanical Identification Methods*; Journal of AOAC International, Vol. 95, pp. 273–285 (2012).

[11] Jeanson, M.L.; Labat, J.-N.; Little, D.P.; *DNA Barcoding: A New Tool for Palm Taxonomists?*; Annals of Botany, Vol. 108, pp. 1445–1451 (2011).

[12] Kress, W.J.; Erickson, D.L.; Jones, F.A.; Swenson, N.G.; Perez, R.; Sanjur, O.; Bermingham, E.; *Plant DNA Barcodes and a Community Phylogeny of a Tropical Forest Dynamics Plot in Panam*a; Proc. Natl. Acad. Sci. U.S.A., Vol. 106, pp. 18621−18626 (2009).
[13] Baker, W.J.; Asumssen, C.B.; Barrow, S.C.; Dransfield, J.; Hedderson, T.A.; *A Phylogenetic Study of the Palm Family (Palmae) Based on Chloroplast DNA Sequences From the trnL-trnF Region*; Plant Syst. Evol., Vol. 219, pp. 111−126 (1999).
[14] Crisp, M.D.; Isagi, Y.; Kato, Y.; Cook, L.G.; Bowman, D.M.J.S.; *Livistona Palms in Australia: Ancient Relics or Opportunistic Immigrants?*; Mol. Phylogenet. Evol., Vol. 54, pp. 512−523 (2010).
[15] Chen, S.; Yao, H.; Han, J.; Liu, C.; Song, J.; Shi, L.; Zhu, Y.; Ma, X.; Gao, T.; Pang, X.; Luo, K.; Li, Y.; Li, X.; Jai, X.; Lin, Y.; Leon, C.; *Validation of the ITS2 Region as a Novel DNA Barcode for Identifying Medicinal Plant Species*; PLoS ONE, Vol. 5, e8613 (2010).

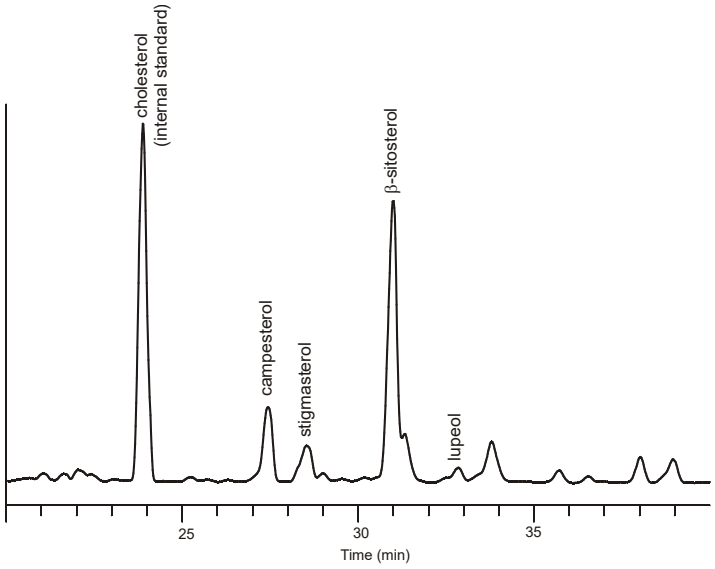
Certificate Revision History: 27 January 2021 (Change of expiration date; removal of certified and reference values for fatty acids due to suspected instability; removal of toxic element analyses and values for As, Cd, Hg, and Pb due to limited data and suspected inhomogeneity of Pb; addition of DNA sequence characterization; editorial changes); **06 June 2017** (Editorial changes); **08 April 2014** (Extension of certification period; editorial changes); **05 January 2009** (Original certificate).

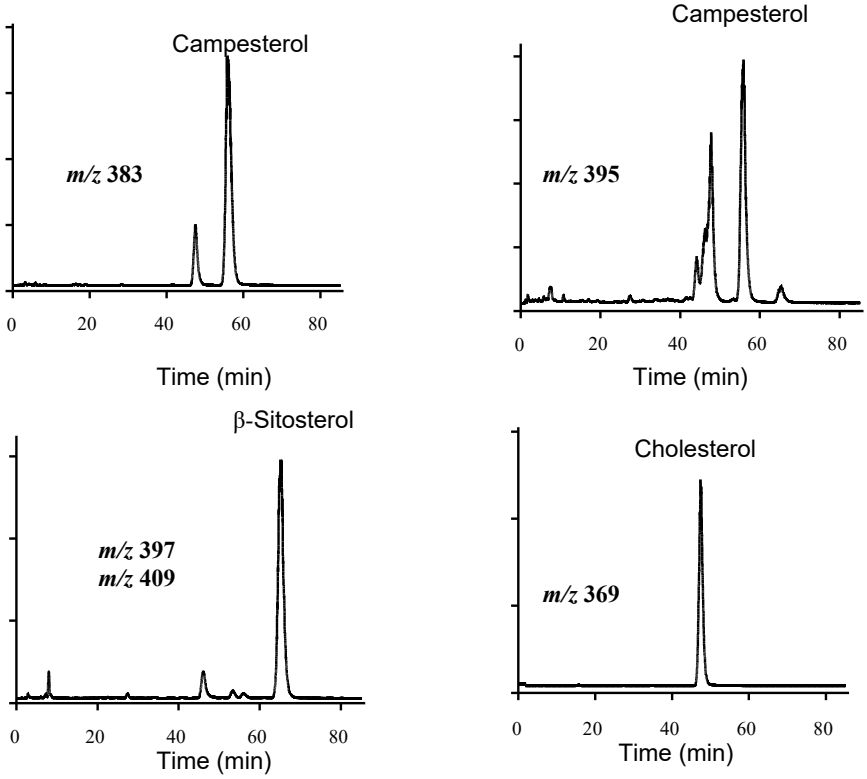
Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; e-mail srminfo@nist.gov; or via the Internet at https://www.nist.gov/srm.

APPENDIX A

Top: Typical chromatogram obtained for the measurement of phytosterols by using GC-FID with a 30 m dimethylpolysiloxane fused silica capillary column (HP-1MS, Agilent Technologies, Wilmington, DE). The column was held isothermally at 250 ºC for 10 min and then temperature programmed at 4 ºC per min to 280 ºC for 22.5 min. The injection port and FID were maintained at 280 ºC. All injections were done in the split mode (1 µL) with helium as a carrier gas at a constant flow rate of 1.2 mL/min.

Bottom: Typical chromatograms for the measurement of phytosterols in SRM 3250 obtained by using LC-MS with a 15 cm ACE C18 column (Advanced Chromatography Technologies, Aberdeen, Scotland). The isocratic mobile phase consisted of 10 % water, 90 % ethanol (volume fractions) at a flow rate of 0.8 mL/min. MS detection conditions were as follows: nebulizer pressure, 276 kPa (40 psi); fragmentor, 80 V; drying gas temperature, 350 °C; drying gas flow rate, 12 L/min; corona current, 8 µA; capillary voltage, 3000 V; and vaporizer temperature, 400 °C.





Appendix B

Reference Samples and Sequences Used in the Specificity Validation Study for SRM 3250

Table B1. Inclusivity Panel for *Serenoa repens*

Serenoa repens	Sample Source[a]	Voucher Information or GenBank Accession #	*psbA-trnH*[b], *trnL-trnF*[b]
1	UCBG	75.0595	+, +
2	UC	1762728	+, +
3	UC	M037645	+, +
4	UCBG	93.1343.300	+, +
5	Texas Natural Supply	unknown	–, +
6	Mt. Rose Herb Co.	M10316	–, +
7	Mars Bazaar	HSAWB	–, +
8	Monterey Spice Company	9E128-543	+, +
9	Kismet Kreations	unknown	–, +
10	AmeriHerb	16390	+, +
11	Frontier Natural Products	unknown	+, +
12	UC	M216776	–, +
13	UC	M216782	–, +
14	AHP	277	–, +
15	UC	582191	–, +

[a] AHP = American Herbal Pharmacopoeia AHP-verified Botanically Identified Reference Material; UC = University Herbarium, University of California, Berkeley; UCBG = University of California Botanical Garden.
[b] Intergenic spacer sequence is included when a plus sign (+) is present or not included when a minus (–) is present.

Table B2. Exclusivity Panel for *Serenoa repens*

Species	Sample Source[a] or Reference	Voucher Information or GenBank Accession #	*psbA-trnH*[b], *trnL-trnF*[b]
Sabal deeringiana	UC	1339550	+, +
Sabal etonia	UCBG	76.0555.300	+, +
Sabal glabra	UC	733395	–, +
Sabal minor	UC	M058245	+, +
Sabal palmetto	UC	1383321	+, +
Sabal palmetto	UC	1348763	–, +
Arenga pinnata	[11]	JF345018	+, –
Arenga westerhoutii	[11]	JF345042	+, –
Bactris barronis	[12]	GQ982157	+, –
Calyptronoma occidentalis	[13]	AJ241313	–, +
Chelyocarpus ulei	[13]	AJ 241254	–, +
Colpothrinax wrightii	[14]	AB522432	–, +
Elaeis oleifera	[12]	GQ982210	+, –
Livistona drudei	[14]	AB522654	–, +
Oenocarpus mapora	[12]	GQ982306	+, –
Prestoea acuminate	[12]	HM446987	+, –
Pritchardia beccariana	[14]	AB522458	–, +
Ravenea rivularis	[14]	AB522464	–, +
Socratea exorrhiza	[12]	GQ982363	+, –
Syagrus smithii	[13]	AJ241310	–, +
Trachycarpus fortune	[14]	AB522460	–, +
Trachycarpus fortune	[15]	GQ435465.1	+, –

[a] UC = University Herbarium, University of California, Berkeley; UCBG = University of California Botanical Garden.
[b] Intergenic spacer sequence is included when a plus sign (+) is present or not included when a minus sign (–) is present.

Appendix C

psbA-trnH DNA Aligned Matrix for *Serenoa repens* and Relatives

```
SRM 3250              TGTCTTAGTGTATATGAATCGTTGAAGGAATGGAGCAATACCCAATATCTTGTTTTAGTT
Serenoa repens        TGTCTTAGTGTATATGAATCGTTGAAGGAATGGAGCAATACCCAATATCTTGTTTTAGTT
Sabal palmetto        TGTCTTAGTGTATATGAATCGTTGAAGGAATGGAGCAATACCCAATATCTTGTTTTAGTT
Sabal deeringiana     TGTCTTAGTGTATATGAATCGTTGAAGGAATGGAGCAATACCCAATATCTTGTTTTAGTT
Sabal etonia          TGTCTTAGTGTATATGAATCGTTGAAGGAATGGAGCAATACCCAATATCTTGTTTTAGTT
Sabal minor           TGTCTTAGTGTATATGAATCGTTGAAGGAATGGAGCAATACCCAATATCTTGTTTTAGTT

SRM 3250              GGGTATTGCTCCATTGTTTGATTCAATAGTGTTTATGCACACAACACATAAGTATTAGTA
Serenoa repens        GGGTATTGCTCCATTGTTTGATTCAATAGTGTTTATGCACACAACACATAAGTATTAGTA
Sabal palmetto        GGGTATTGCTCCATTGTTTGATTCAATAGTGTTT-TGCACATAACACATAAGTA------
Sabal deeringiana     GGGTATTGCTCCATTGTTTGATTCAATAGTGTTT-TGCACATAACACATAAGTA------
Sabal etonia          GGGTATTGCTCCATTGTTTGATTCAATAGTGTTT-TGCACATAACACATAAGTA------
Sabal minor           GGGTATTGCTCCATTGTTTGATTCAATAGTGTTT-TGCACATAACACATAAGTA------

SRM 3250              TAGCACATAAGTATTAGTAATGAATGATAAGTACTTTTTTAGTATTTTTTTT--------
Serenoa repens        TAGCACATAAGTATTAGTAATGAATGATAAGTACTTTTTTAGTATTTTTTTT--------
Sabal palmetto        ------------------AATGAATGATAAGTACTTTTTTCTTATTTTTTTTTATTTTAC
Sabal deeringiana     ------------------AATGAATGATAAGTACTTTTTTCTTATTTTTTTTTATTTTAC
Sabal etonia          ------------------AATGAATGATAAGTACTTTTTTCTTATTTTTTTTTATTTTAC
Sabal minor           ------------------AATGAATGATAAGTACTTTTTTCTTATTTTTTTTTATTTTAC

SRM 3250              ----------TTTATATTAATAATATTTCTATTAATATTTAATATTCAAAAATATTATTT
Serenoa repens        ----------TTTATATTAATAATATTTCTATTAATATTTAATATTCAAAAATATTATTT
Sabal palmetto        TTTTTATTTATTTCTATTAATAATATTTCTATTAATATTTAATATTCAAAAATATTATGA
Sabal deeringiana     TTTTTATTTATTTCTATTAATAATATTTCTATTAATATTTAATATTCAAAAATATTATGA
Sabal etonia          TTTTTATTTATTTCTATTAATAATATTTCTATTAATATTTAATATTCAAAAATATTATGA
Sabal minor           TTTTTATTTATTTCTATTAATAATATTTCTATTAATATTTAATATTCAAAAATATTATGA

SRM 3250              AAATTTAAATAATTTAACGACGAGATTTATTGTCGTTTCTTGCATGTCTCGCGAAAGTCA
Serenoa repens        AAATTTAAATAATTTAACGACGAGATTTATTGTCGTTTCTTGCATGTCTCGCGAAAGTCA
Sabal palmetto        AAATTTCAATAATTTAACGACGAGATTTATTGTCGTTTCTTGCATGTCTCGCGAAAGTCA
Sabal deeringiana     AAATTTCAATAATTTAACGACGAGATTTATTGTCGTTTCTTGCATGTCTCGCGAAAGTCA
Sabal etonia          AAATTTCAATAATTTAACGACGAGATTTATTGTCGTTTCTTGCATGTCTCGCGAAAGTCA
Sabal minor           AAATTTCAATAATTTAACGACGAGATTTATTGTCGTTTCTTGCATGTCTCGCGAAAGTCA

SRM 3250              GAGTAGGCGCGAATTCTCCCAATTTGTGACCTACCATACGATCTGTTATATAAATAGGTA
Serenoa repens        GAGTAGGCGCGAATTCTCCCAATTTGTGACCTACCATACGATCTGTTATATAAATAGGTA
Sabal palmetto        GAGTAGGCGCGAATTCTCCCAATTTGTGACCTACCATACGATCTGTTATATAAATAGGTA
Sabal deeringiana     GAGTAGGCGCGAATTCTCCCAATTTGTGACCTACCATACGATCTGTTATATAAATAGGTA
Sabal etonia          GAGTAGGCGCGAATTCTCCCAATTTGTGACCTACCATACGATCTGTTATATAAATAGGTA
Sabal minor           GAGTAGGCGCGAATTCTCCCAATTTGTGACCTACCATACGATCTGTTATATAAATAGGTA

SRM 3250              AATTTTCCTTTCCATTATGAATAGCGATTGTATGGCCAATCATTGTGGGTATAATGGTAG
Serenoa repens        AATTTTCCTTTCCATTATGAATAGCGATTGTATGGCCAATCATTGTGGGTATAATGGTAG
Sabal palmetto        AATTTTCCTTTCCATTATGAATAGCGATTGTATGGCCAATCATTGTGGGTATAATGGTAG
Sabal deeringiana     AATTTTCCTTTCCATTATGAATAGCGATTGTATGGCCAATCATTGTGGGTATAATGGTAG
Sabal etonia          AATTTTCCTTTCCATTATGAATAGCGATTGTATGGCCAATCATTGTGGGTATAATGGTAG
Sabal minor           AATTTTCCTTTCCATTATGAATAGCGATTGTATGGCCAATCATTGTGGGTATAATGGTAG

SRM 3250              ATGCCCGAGACCAAGTTACTATTATTTCTTTCTCCTCCCTCATGTTGAGTTTTTCAATTT
Serenoa repens        ATGCCCGAGACCAAGTTACTATTATTTCTTTCTCCTCCCTCATGTTGAGTTTTTCAATTT
Sabal palmetto        ATGCCCGAGACCAAGTTACTATTATTTCTTTCTCCTCCCTCATGTTGAGTTTTTCAATTT
Sabal deeringiana     ATGCCCGAGACCAAGTTACTATTATTTCTTTCTCCTCCCTCATGTTGAGTTTTTCAATTT
Sabal etonia          ATGCCCGAGACCAAGTTACTATTATTTCTTTCTCCTCCCTCATGTTGAGTTTTTCAATTT
Sabal minor           ATGCCCGAGACCAAGTTACTATTATTTCTTTCTCCTCCCTCATGTTGAGTTTTTCAATTT

SRM 3250              TTCCCGATAAATGATTAGCTACAAAAGGATTTTTTTTTTAGTGAACGTGTCACAGCTGATT
Serenoa repens        TTCCCGATAAATGATTAGCTACAAAAGGATTTTTTTTTTAGTGAACGTGTCACAGCTGATT
Sabal palmetto        TTCCCGATAAATGATTAGCTACAAAAGGATTTTTTTTTTAGTGAACGTGTCACAGCTGATT
Sabal deeringiana     TTCCCGATAAATGATTAGCTACAAAAGGATTTTTTTTTTAGTGAACGTGTCACAGCTGATT
Sabal etonia          TTCCCGATAAATGATTAGCTACAAAAGGATTTTTTTTTTAGTGAACGTGTCACAGCTGATT
Sabal minor           TTCCCGATAAATGATTAGCTACAAAAGGATTTTTTTTTTAGTGAACGTGTCACAGCTGATT

SRM 3250              ACTCCTTTTTTTTTTTACATTTTAAAGATTGGCATTCTATGTCCAATATCTCGATCTAAGT
Serenoa repens        ACTCCTTTTTTTTTTTACATTTTAAAGATTGGCATTCTATGTCCAATATCTCGATCTAAGT
Sabal palmetto        ACTCCTTTTTTTTTTTACATTTTAAAGATTGGCATTCTATGTCCAATATCTCGATCTAAGT
Sabal deeringiana     ACTCCTTTTTTTTTTTACATTTTAAAGATTGGCATTCTATGTCCAATATCTCGATCTAAGT
Sabal etonia          ACTCCTTTTTTTTTTTACATTTTAAAGATTGGCATTCTATGTCCAATATCTCGATCTAAGT
Sabal minor           ACTCCTTTTTTTTTTTACATTTTAAAGATTGGCATTCTATGTCCAATATCTCGATCTAAGT
```

```
SRM 3250                ATGGAGGTCAGAATAAATACAATAATGATGAATGGAAAA
Serenoa repens          ATGGAGGTCAGAATAAATACAATAATGATGAATGGAAAA
Sabal palmetto          ATGGAGGTCGGAATAAATACAATAATGATGAATGGAAAA
Sabal deeringiana       ATGGAGGTCGGAATAAATACAATAATGATGAATGGAAAA
Sabal etonia            ATGGAGGTCGGAATAAATACAATAATGATGAATGGAAAA
Sabal minor             ATGGAGGTCGGAATAAATACAATAATGATGAATGGAAAA
```

Figure C1. *psbA-trnH* DNA Aligned Matrix for *Serenoa repens* and Relatives. The results from the chloroplast region demonstrates that this region does distinguish this species from its relatives and does authenticate *Serenoa repens.* The individual bases are represented as A = Adenine, T = Thymine, G = Guanine, and C = Cystosine. A " - " = missing data. The confidence estimate for the species identification of SRM 3250 as *Serenoa repens* is Most Confident (0).

trnL-trnF DNA Aligned Matrix for *Serenoa repens* and Relatives

```
SRM 3250              GTCCATTTTACTTCCTAACTATTTATCCTCTTTTTTTCATCAGTGGTTCAAACAAAATTC
Serenoa repens        GTCCATTTTACTTCCTAACTATTTATCCTCTTTTTTTCATCAGTGGTTCAAACAAAATTC
Sabal palmetto        GTCCATTTTACTTCCTAACTATTTATCCTCTTTTTTTCATCAGTGGTTCAAACAAAATTC
Sabal glabra          GTCCATTTTACTTCCTAACTATTTATCCTCTTTTTTTCATCAGTGGTTCAAACAAAATTC
Sabal deeringiana     GTCCATTTTACTTCCTAACTATTTATCCTCTTTTTTTCATCAGTGGTTCAAACAAAATTC
Sabal minor           GTCCATTTTACTTCCTAACTATTTATCCTCTTTTTTTCATCAGTGGTTCAAACAAAATTC
Sabal etonia          GTCCATTTTACTTCCTAACTATTTATCCTCTTTTTTTCATCAGTGGTTCAAACAAAATTC

SRM 3250              ACTATCTTTCTTTCTCATTCACTCTACTCTTTCACAAATGGATCCGAAGAGAAATCTTTG
Serenoa repens        ACTATCTTTCTTTCTCATTCACTCTACTCTTTCACAAATGGATCCGAAGAGAAATCTTTG
Sabal palmetto        ACTATCTTTCTTTCTCATTCACTCTACTCTTTCACAAAAGGATCCGAAGAGAAATCTTTG
Sabal glabra          ACTATCTTTCTTTCTCATTCACTCTACTCTTTCACAAAAGGATCCGAAGAGAAATCTTTG
Sabal deeringiana     ACTATCTTTCTTTCTCATTCACTCTACTCTTTCACAAAAGGATCCGAAGAGAAATCTTTG
Sabal minor           ACTATCTTTCTTTCTCATTCACTCTACTCTTTCACAAAAGGATCCGAAGAGAAATCTTTG
Sabal etonia          ACTATCTTTCTTTCTCATTCACTCTACTCTTTCACAAAAGGATCCGAAGAGAAATCTTTG

SRM 3250              GATCTTATCCCAATTTGGATAGATACGATACCTGTACAAATGAACATATATGGGCAATGA
Serenoa repens        GATCTTATCCCAATTTGGATAGATACGATACCTGTACAAATGAACATATATGGGCAATGA
Sabal palmetto        GATCTTATCCCAATTTGGATAGATACGATACTTGTACAAATGAACATATATGGGCAAGGA
Sabal glabra          GATCTTATCCCAATTTGGATAGATACGATACTTGTACAAATGAACATATATGGGCAAGGA
Sabal deeringiana     GATCTTATCCCAATTTGGATAGATACGATACTTGTACAAATGAACATATATGGGCAAGGA
Sabal minor           GATCTTATCCCAATTTGGATAGATACGATACTTGTACAAATGAACATATATGGGCAAGGA
Sabal etonia          GATCTTATCCCAATTTGGATAGATACGATACTTGTACAAATGAACATATATGGGCAAGGA

SRM 3250              ATCTCTATTATTGAATCATTCACAGTCCATATCATTATCCTTACATTTATTAGATAAAAT
Serenoa repens        ATCTCTATTATTGAATCATTCACAGTCCATATCATTATCCTTACATTTATTAGATAAAAT
Sabal palmetto        ATCTCTATTATTGAATCATTCACAGTCCATATCATTATCCTTACATTTTTTAGATAAAAT
Sabal glabra          ATCTCTATTATTGAATCATTCACAGTCCATATCATTATCCTTACATTTTTTAGATAAAAT
Sabal deeringiana     ATCTCTATTATTGAATCATTCACAGTCCATATCATTATCCTTACATTTTTTAGATAAAAT
Sabal minor           ATCTCTATTATTGAATCATTCACAGTCCATATCATTATCCTTACATTTTTTAGATAAAAT
Sabal etonia          ATCTCTATTATTGAATCATTCACAGTCCATATCATTATCCTTACATTTTTTAGATAAAAT

SRM 3250              TTTTTAATACTTTACTTTATTTAATACTTTACTTTATTTAGATTTAGATTTAGTCCCTTT
Serenoa repens        TTTTTAATACTTTACTTTATTTAATACTTTACTTTATTTAGATTTAGATTTAGTCCCTTT
Sabal palmetto        TTTTTAATACTTTATTTTATTTAATACTTTACTTTATTTAGATTTAG------TCCCTTT
Sabal glabra          TTTTTAATACTTTATTTTATTTAATACTTTACTTTATTTAGATTTAG------TCCCTTT
Sabal deeringiana     TTTTTAATACTTTATTTTATTTAATACTTTACTTTATTTAGATTTAG------TCCCTTT
Sabal minor           TTTTTAATACTTTATTTTATTTAATACTTTACTTTATTTAGATTTAG------TCCCTTT
Sabal etonia          TTTTTAATACTTTATTTTATTTAATACTTTACTTTATTTAGATTTAG------TCCCTTT

SRM 3250              AATTGACATAGATACAAGTACTCTACTAGGATGATGCACGGGAAATGGTCGGGATAGCTC
Serenoa repens        AATTGACATAGATACAAGTACTCTACTAGGATGATGCACGGGAAATGGTCGGGATAGCTC
Sabal palmetto        AATTGACATAGATACAAGTACTCTACTAGGATGATGCACGGGAAATGGTCGGGATAGCTC
Sabal glabra          AATTGACATAGATACAAGTACTCTACTAGGATGATGCACGGGAAATGGTCGGGATAGCTC
Sabal deeringiana     AATTGACATAGATACAAGTACTCTACTAGGATGATGCACGGGAAATGGTCGGGATAGCTC
Sabal minor           AATTGACATAGATACAAGTACTCTACTAGGATGATGCACGGGAAATGGTCGGGATAGCTC
Sabal etonia          AATTGACATAGATACAAGTACTCTACTAGGATGATGCACGGGAAATGGTCGGGATAGCTC

SRM 3250              AGTTGGTAGAGCAGAGGACT
Serenoa repens        AGTTGGTAGAGCAGAGGACT
Sabal palmetto        AGTTGGTAGAGCAGAGGACT
Sabal glabra          AGTTGGTAGAGCAGAGGACT
Sabal deeringiana     AGTTGGTAGAGCAGAGGACT
Sabal minor           AGTTGGTAGAGCAGAGGACT
Sabal etonia          AGTTGGTAGAGCAGAGGACT
```

Figure D1. *trnL-trnF* DNA Aligned Matrix for *Serenoa repens* and Relatives. The results from the chloroplast region demonstrates that this region does distinguish this species from its relatives and does authenticate *Serenoa repens.* The individual bases are represented as A = Adenine, T = Thymine, G = Guanine, C = Cystosine. A "-" = missing data. The confidence estimate for the species identification of SRM 3250 as *Serenoa repens* is Most Confident (0).