National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 3262

St. John's Wort (*Hypericum perforatum L.*) Aerial Parts

This Standard Reference Material (SRM) is intended primarily for use in validating analytical methods for the determination of chlorogenic acid, flavonoids, naphthodianthrones, and toxic elements in *Hypericum perforatum L.* and similar materials. This SRM can 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 3262 consists of five heat-sealed aluminized pouches, each containing approximately 3.3 g of material.

The development of SRM 3262 was through 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 (FDA) Center for Drug Evaluation and Research (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), AuthenTechnologies (Richmond, CA), and American Herbal Pharmacopoeia (Scotts Valley, CA).

Taxonomic Identification: The taxonomic identity is *Hypericum perforatum L.* established through identification by a trained botanist using an herbarium specimen from original material and from associated DNA sequence analysis from botanically authenticated *Hypericum perforatum L.* 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 *Hypericum perforatum L.*

Certified Mass Fraction Values: Certified mass fraction values for toxic elements in SRM 3262, reported on a dry-mass basis, 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 [3]. Analyses for value assignment were performed by NIST. Certified values were calculated as the mean of the mean values from NIST methods. The associated uncertainties are expressed at an approximately 95 % level of confidence [4–6].

Reference Mass Fraction Values: Reference mass fraction values, reported on a dry-mass basis, are provided for chlorogenic acid, flavonoids, and naphthodianthrones (Table 4) and lead (Table 5). 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 [3] 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 were derived from results reported by NIST.

Expiration of Certification: The certification of **SRM 3262** is valid, within the measurement uncertainty specified, until **01 March 2026**, 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

Coordination of the technical measurements leading to the certification of this SRM was performed by C.A. Rimmer, L.J. Wood of the NIST Chemical Sciences Division, and K.E. Sharpless formerly of the NIST Chemical Sciences Division.

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Certificate Issue Date: 14 September 2016

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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.

Support for the development of SRM 3262 was provided in part by the NIH-ODS and the FDA CDER. Technical consultation from these agencies was provided by J.M. Betz (NIH-ODS) and A. NguyenPho (FDA CDER). Acquisition and preparation of the material were coordinated by A. NguyenPho and K.E. Sharpless.

Analytical measurements at NIST were performed by B.L. Catron, K.D. Chieh, P.B. Howell, S.E. Long, K.E. Murphy, M.A. Nelson, R.L. Paul, M.M. Phillips, and L.J. Wood of the NIST Chemical Sciences Division. Molecular approach for species identity was coordinated and performed by D.H. Reynaud of AuthenTechnologies, with two collaborating laboratories for sequencing analyses: University of California Berkeley DNA Sequencing Facility (Berkeley, CA) and Sequetech, Inc. (Mountain View, CA). Technical consultation was provided by J.M. Betz (NIH-ODS), J. Harnly (USDA-ARS), R. Upton (American Herbal Pharmacopoeia), and W. Applequist (Missouri Botanical Garden, St. Louis, MO).

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 TO USERS: SRM 3262 IS INTENDED FOR RESEARCH USE, 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 its original unopened packets, until required for use. For elemental analyses, the packet can be resealed and test portions removed and analyzed until the material reaches its expiration date. For chlorogenic acid, flavonoids, and naphthodianthrones analysis, the packet can be resealed and test portions removed and analyzed for up to two months after opening.

Use: Prior to use, the contents of the packet should be mixed thoroughly. Allow the contents to settle for one minute prior to opening to minimize the loss of fine particles. To relate analytical determinations to the certified or reference values in this Certificate of Analysis, the minimum test portion mass indicated in the "Source, Preparation, and Analysis" section for each group of analytes below 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 [7]. The moisture conversion factor can be used for the sample(s) when using an unopened packet for the first time. If using a previously opened and resealed packet, sample(s) need to be dried using one of the recommended techniques (see "Determination of Moisture").

Determination of Moisture: Moisture content of SRM 3262 was determined at NIST by (1) drying over magnesium perchlorate in a desiccator at room temperature for 14 d and (2) drying for 2 h in a forced-air oven at 70 °C. Unweighted results obtained using both techniques were averaged to determine a conversion factor of (0.9512 ± 0.0022) 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 (coverage factor $k = 2$). A relative uncertainty component for the conversion factor (0.11 %) 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: The SRM is a cultivated, dried plant material of St. John's wort (*Hypericum perforatum L.*) purchased from a commercial source. The material was received as whole stalks; the leaves and petioles were stripped off, ground in a blender, and sieved to 180 μm (80 mesh). The material was shipped to High-Purity Standards (Charleston, SC) where it was blended, aliquoted, and heat-sealed inside nitrogen-flushed 4 mil polyethylene bags, which were then sealed inside nitrogen-flushed aluminized plastic bags along with two packets of silica gel each. After packaging, the material was irradiated (Neutron Products, Inc., Dickerson, MD) by ^{60}Co to an absorbed dose of 27.5 kGy to 33.5 kGy.

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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.

Analytical Approach for Determination of Chlorogenic Acid, Flavonoids, and Naphthodianthrones: Value assignment of the mass fractions of chlorogenic acid, flavonoids, and naphthodianthrones in SRM 3262 was based on measurements provided by NIST using liquid chromatography (LC) with absorbance and/or fluorescence detection.

NIST Analyses for Chlorogenic Acid, Rutin, Hyperoside, Quercitrin, Hypericin, and Pseudohypericin: The mass fractions of chlorogenic acid, rutin, hyperoside, quercitrin, hypericin, and pseudohypericin were determined by LC with absorbance and/or fluorescence detection from duplicate, nominal 1.0 g test portions taken from 10 packets of SRM 3262. Test portions were combined with a measured mass of internal standard solution (rhodamine 6G), 1 g hydromatrix, and methanol, placed in a Pyrex Soxhlet extraction thimble and allowed to reflux for 24 h before analysis by LC. A C18 column and a gradient method with a mobile phase consisting of (A) aqueous 0.5 % triethylamine (volume fractions) adjusted to pH 4.5 with acetic acid and (B) acetonitrile were used. Absorbance at 340 nm was monitored for detection of chlorogenic acid, rutin, hyperoside, and quercitrin, and absorbance at 590 nm was monitored for detection of hypericin and pseudohypericin using a photodiode array detector. Hypericin and pseudohypericin were also monitored by fluorescence at an excitation wavelength of 470 nm and an emission wavelength of 590 nm. Additional method details and a typical chromatogram are provided in Appendix E. Calibrants were prepared gravimetrically at levels intended to approximate levels of the analytes in the extracts of the SRM. The purity of neat calibrant materials for chlorogenic acid, hyperoside, quercitrin, hypericin, and pseudohypericin was determined at NIST by quantitative proton nuclear magnetic resonance spectroscopy (qNMR). The purity of the neat calibrant material for rutin was determined at NIST by LC with absorbance detection. A single internal standard solution was used for calibrants and samples.

Analytical Approach for Determination of Elements: Value assignment of the mass fractions of the elements in SRM 3262 was based on measurements provided by NIST using isotope dilution/cold vapor inductively coupled plasma mass spectrometry (ID/CV ICP-MS), inductively coupled plasma mass spectrometry (ICP-MS), isotope dilution inductively coupled plasma mass spectrometry (ID ICP-MS), and instrumental neutron activation analysis (INAA).

NIST Analyses for As by INAA: The mass fraction of arsenic was determined by INAA from duplicate, nominal 0.3 g test portions taken from ten packets of SRM 3262. Test portions were packaged individually in clean polyethylene bags along with gravimetric standards made from SRM 3103a, lot #100818, Arsenic (As) Standard Solution and were distributed among four irradiation vessels. Each vessel was irradiated for 2 h at 20 MW and rotated end-over-end after 1 h to ensure a uniform neutron flux. Test portions were allowed to decay 5 d to 6 d after which test portions were counted for 5 h. The 559 keV line from decay of ^{76}As was used for quantification.

NIST Analyses for As by ICP-MS: The mass fraction of arsenic was determined by ICP-MS from duplicate, nominal 0.5 g test portions taken from six packages of SRM 3262. Samples were digested in closed vessels using nitric acid in a microwave sample preparation system. Quantification was based on the method of standard additions using spectrometric single element standard solution SRM 3103a, lot #100818.

NIST Analyses for Cd and Pb by ID ICP-MS: Mass fractions of cadmium and lead in SRM 3262 were determined by ID ICP-MS from duplicate, nominal 0.7 g test portions taken from each of six (Pb) to twelve (Cd) packets of SRM 3262. Samples were spiked with isotopically enriched ^{206}Pb and ^{111}Cd and were digested in nitric acid using a microwave sample preparation system. Digests were transferred to plastic bottles and diluted with the appropriate volume of 2 % (volume fraction) nitric acid. Lead was measured by ICP-MS in standard mode, whereas cadmium was measured in collision cell/kinetic energy discrimination mode. The use of ID ICP-MS for the accurate measurement of Cd has been described in detail [8]. Cadmium standards were prepared from SRM 3108 Cadmium Standard Solution and high purity Cd metal (99.999+ percent purity) whose source is SRM 746 Cadmium-Vapor Pressure. Lead standards were prepared from SRM 3128 Lead (Pb) Standard Solution, and from high purity Pb metal (99.999 percent purity) obtained from Johnson Matthey (London, UK). The standards were used to calibrate the amount of added ^{111}Cd and ^{206}Pb *via* reverse ID ICP-MS [8].

NIST Analyses for Hg by ID/CV ICP-MS: The mass fraction of mercury in SRM 3262 was determined by ID/CV ICP-MS from single, nominal 0.2 g test portions taken from each of eight packets of SRM 3262. Samples were spiked with isotopically enriched ^{201}Hg and were digested in a nitric acid/hydrochloric acid mixture using a microwave sample preparation system. Quantification was based on the method of isotope dilution analysis monitoring the ^{201}Hg/^{202}Hg isotope ratios [9].

ASSIGNMENT OF IDENTITY

Molecular Approach for Species Identity: Sanger sequencing was used on three independent chloroplast gene regions, *trnL-trnF* intergenic region [10,11], *rbcL* intron [12], and the nuclear ribosomal gene internal transcribed spacer (ITS) sequence for authentication of SRM 3262. Complete sequencing of the strands of DNA for the three 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 [13]. 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 A 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 B, C and D, were used to determine the species identity of SRM 3262 and to estimate the confidence (as described below). The confidence levels for both the *trnL-trnF* intergenic region, the *rbcL* intron, and the ITS sequence were "Most Confident" (0).

The taxonomic identification is *Hypericum perforatum L.* and the associated chloroplast DNA sequences from the *trnL-trnF* intergenic spacer and *rbcL* intron regions and from the nuclear ribosomal gene internal transcribed spacer (ITS) sequence 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 *Hypericum perforatum L.* Chloroplast and nuclear ribosomal DNA sequences from botanically authenticated *Hypericum perforatum L.* 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 3262, 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 B, 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 *trnL-trnF* intergenic spacer, *rbcL* intron region, and ITS 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 chlorogenic acid, flavonoids, naphthodianthrones, and elements was assessed at NIST using the methods and test portion sizes described above. Based on statistical analysis of analytical results from NIST, and allowing for potential uncertainty for material heterogeneity, the uncertainty for cadmium and lead incorporate an additional component for possible heterogeneity. The homogeneity of the DNA sequences was evaluated by sequencing the DNA from multiple samples from three randomly-selected packets, revealing the degree of homogeneity in DNA sequence or species identity. The DNA data were homogeneous.

NIST strives to maintain the SRM inventory supply, but NIST cannot guarantee the continued or continuous supply of any specific SRM. Accordingly, NIST encourages the use of this SRM as a primary benchmark for the quality and accuracy of the user's in-house reference materials and working standards. As such, the SRM should be used to validate the more routinely used reference materials in a laboratory. Comparisons between the SRM and in-house reference materials or working measurement standards should take place at intervals appropriate to the conservation of the SRM and the stability of relevant in-house materials. For further guidance on how this approach can be implemented, contact NIST by email at srms@nist.gov.

Certified Mass Fraction Values for Selected Elements: Each certified mass fraction value, reported on a dry-mass basis, is the combined mean from the means of results from analyses provided by NIST. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = k u_c$, where u_c incorporates the observed difference between the results from the methods and their respective uncertainties consistent with the ISO/JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [4–6]. The uncertainty for Cd incorporates an uncertainty component for possible heterogeneity. The measurand is the total mass fraction for each element in St. John's Wort (*Hypericum perforatum L.*) Aerial Parts as listed in Table 3. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per kilogram) on a dry-mass basis.

Table 3. Certified Mass Fraction Values for Elements in SRM 3262

	Mass Fraction (mg/kg)	Coverage Factor, k
Arsenic (As)[a,b]	0.152 ± 0.014	2.00
Cadmium (Cd)[c]	0.3638 ± 0.0094	2.13
Mercury (Hg)[d]	0.01479 ± 0.00043	2.36

[a] ICP-MS
[b] RNAA
[c] ID ICP-MS
[d] ID/CV ICP/MS

Reference Mass Fraction Values for Chlorogenic Acid, Flavonoids, and Naphthodianthrones: Each reference mass fraction value, reported on a dry-mass basis, is the mean of results from NIST analyses by LC with absorbance and/or fluorescence detection. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = k u_c$, where u_c represents the combined uncertainty consistent with the ISO/JCGM Guide and k is a coverage factor corresponding to approximately 95 % confidence [4]. The measurand is the mass fraction for each analyte in St. John's Wort (*Hypericum perforatum L.*) Aerial Parts as listed in Table 4 as determined by the indicated method. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per gram) on a dry-mass basis.

Table 4. Reference Mass Fraction Values for Chlorogenic Acid, Flavonoids, and Naphthodianthrones in SRM 3262

	Mass Fraction (mg/g)	Coverage Factor, k
Chlorogenic Acid[a]	0.1620 ± 0.0078	2.11
Rutin[a]	5.31 ± 0.12	2.11
Hyperoside[a]	5.28 ± 0.11	2.11
Quercitrin[a]	1.035 ± 0.032	2.11
Hypericin[a,b]	0.542 ± 0.019	2.00
Pseudohypericin[a,b]	0.747 ± 0.021	2.00

[a] LC-absorbance
[b] LC-fluorescence

Reference Mass Fraction Value for Lead: The reference mass fraction value, reported on a dry-mass basis, is the mean of results from NIST analyses by ID ICP-MS. The uncertainty provided with the value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = k u_c$, where u_c represents the combined uncertainty consistent with the ISO/JCGM Guide and k is a coverage factor corresponding to approximately 95 % confidence [4]. The uncertainty for Pb incorporates an uncertainty component for possible heterogeneity. The measurand is the mass fraction for lead in St. John's Wort (*Hypericum perforatum L.*) Aerial Parts as listed in Table 5 as determined by the indicated method. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per kilogram) on a dry-mass basis.

Table 5. Reference Mass Fraction Value for Lead in SRM 3262

	Mass Fraction (mg/kg)	Coverage Factor, k
Lead (Pb)[a]	0.98 ± 0.14	2.23

[a] ID ICP-MS

REFERENCES

[1] Certified data can be downloaded at https://www-s.nist.gov/srmors/view_detail.cfm?srm=3262.
 — The certified sequence data file for *trnL-trnF* intergenic region is:
 SRM3262 trnL-trnF Sequence_v1.FASTA.
 — The certified confidence estimates for every base of *trnL-trnF* intergenic region sequence data file is:
 SRM3262 trnL-trnF Nucleotide Confidence_v1.TXT.
 — The certified sequence data file for *rbcL* intron is:
 SRM3262 rbcL Sequence_v1.FASTA.
 — The certified confidence estimates for every base of *rbcL-trnF* intron region sequence data file is:
 SRM3262 rbcL Nucleotide Confidence_v1.TXT.
 — The certified sequence data file for ITS sequence is:
 SRM3262 ITS Sequence_v1.FASTA.
 — The certified confidence estimates for every base of ITS sequence data file is:
 SRM3262 ITS Nucleotide Confidence_v1.TXT.

[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 Sep 2016).

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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 http://www.nist.gov/srm.

Reference Samples and Sequences Used in the Specificity Validation Study for SRM 3262

Table A1. Inclusivity Panel for *Hypericum perforatum L.*

Hypericum perforatum L.	Sample Source[a]	Voucher Information or GenBank Accession #	ITS[b], *trnL-trnF*[c], *rbcL*[d]
1	UCBG	78.0502	+, +, +
2	UC	BC1783971	+, −, +
3	UC	BC114533	+, +, +
4	AHP	2690	+, +, +
5	AHP	992	+, +, +
6	AHP	2870	+, +, +
7	AHP	2865	+, +, −
8	AHP	2877	+, +, +
9	AHP	771b	+, +, +
10	AHP	989	+, +, +
11	AHP	993	+, +, +
12	AHP	965	+, +, +
13	AHP	990	+, +, −
14	AHP	988	+, +, −
15	AHP	2657	−, +, +

[a] AHP = American Herbal Pharmacopoeia (AHP-Verified Botanical Identity Reference Material); AT = AuthenTechnologies Herbarium; UC = University Herbarium, University of California, Berkeley; UCBG = University of California Botanical Garden.
[b] Internal Transcribed Spacer Sequence is included when a plus sign (+) is present.
[c] Intergenic spacer sequence is included when a plus sign (+) is present.
[d] Intron sequence is included when a plus sign (+) is present.

Table A2. Exclusivity Panel for *Hypericum perforatum L.*

Species	Sample Source[a] or Reference	Voucher Information or GenBank Accession #	ITS[b], *trnL-trnF*[c], *rbcL*[d]
Hypericum hirsutum	AHP	994	+, +, +
Hypericum punctatum Lam	UC	965237	?, −, +
Hypericum punctatum Lam	UC	1358607	?, +, +
Hypericum punctatum Lam	UC	871976	?, −, +
Hypericum maculatum Crantz	UC	1474097	+, −, +
Hypericum maculatum Crantz	UC	1397220	+, −, +
Hypericum maculatum Crantz	UC	M211846	+, −, +
Hypericum majus	UC	M211846	+, −, +
Hypericum undulatum	AHP	995	+, +, +
Hypericum sp.	UC	M180044	+, ?, ?

[a] AHP = American Herbal Pharmacopoeia (AHP-Verified Botanical Identity Reference Material); UC = University Herbarium, University of California, Berkeley.
[b] Internal Transcribed Spacer Sequence is included when a plus sign (+) is present, not included when a minus (−) is present, or unknown when a (?) is present.
[c] Intergenic spacer sequence is included when a plus sign (+) is present, not included when a minus (−) is present, or unknown when a (?) is present.
[d] Intron sequence is included when a plus sign (+) is present, not included when a minus (−) is present, or unknown when a (?) is present.

trnL-trnF DNA Aligned Matrix for *Hypericum perforatum L.* and Relatives

```
SRM 3262             GGTAATCCTGAACCAAATCCGGCTTTCCGAAAACAAAGAAAGATTCATAAACAG---AAA
Hypericum perforatum GGTAATCCTGAACCAAATCCGGCTTTCCGAAAACAAAGAAAGATTCATAAACAG---AAA
Hypericum punctatum  GGTAATCCTGAGCCAAATCCGGCTTTCCGAAAACAAAGAAAGATTCATAAACAG---AAA
Hypericum maculatum  GGTAATCCTGARCCAAATCCGGCTTTCCGAAAACAAAGAAAGATTCATAAACAG---AAA
Hypericum undulatum  ---AATCCTGAGCCAAATCCGGCTTTCCGAAAACAAAGAAAGATTCATAAACAGAAAAAA
Hypericum hirsutum   GGCAATCCTGAGCCAAATCCGGCTTTCCGAAAACAAAGAAAGATTCATAAACAG---AAA
Hypericum majus      ------------------------------------------------------------

SRM 3262             AAAAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAAAAAATGAAGTGGCTTGCAA
Hypericum perforatum AAAAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAAAAAATGAAGTGGCTTGCAA
Hypericum punctatum  AAAAUAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAAAAAATGAAGTGGATTGCGA
Hypericum maculatum  AAAAAAGGATAGGTGCARAGACTCAATGGAAGCTGTTCTAAAAAATGAAGTGGCTTGCRA
Hypericum undulatum  AAAAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAAAAAATGAAGTGGCTTGCGA
Hypericum hirsutum   AAAAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAAGAAATGAAGTGGATTGCAA
Hypericum majus      -----------------GACTCAATGGAAGTTATTCTAAAAAATGAAGTGGATTGCGA

SRM 3262             TTAGTAAAAAGGATAAACTATACAT-------ATATGTGACAAATGTTAATCTACGTATT
Hypericum perforatum TTAGTAAAAAGGATAAACTATACAT-------ATATGTGACAAATGTTAATCTACGTATT
Hypericum punctatum  TTAGTAAAAAGGATAAACTATACATATATGTGATATGTGACAAATATAAATCTACRTATT
Hypericum maculatum  TTAGTAAAAAGGATAAACTATACAT-------ATATGTGACAAATGTTAATCTACGTATT
Hypericum undulatum  TTAGTAAAAAGGATAAACTATACAT-------ATATGTGACAAATGTTAATCTACGTATT
Hypericum hirsutum   TTAGTAAAAAGGATAAACTATACAT------AGATGTGTATATATACATCTACGTATT
Hypericum majus      TTAGTAAAAAGGATAAACCATACAT------ATATATGACAAATATACATCTACGTATT

SRM 3262             AACATACTATATCAAA--AGATTACAGTCTGGTCTGATAAATAACTGATAAATTGCACGA
Hypericum perforatum AACATACTATATCAAA--AGATTACAGTCTGGTCTGATAAATAACTGATAAATTGCACGA
Hypericum punctatum  AACATACTATATCAAA--AGATTACARTCTGGTCTGATAAATAACTGATAAATTGCACGA
Hypericum maculatum  AACATACTATATCAAA--AGATTACAGTCTGGTCTGAWAAATAACTGATAAATTGCACRA
Hypericum undulatum  AACATACTATATCAAA--AGATTACAGTCTGGTCTGATAAATAACTGATAAATTGCACGA
Hypericum hirsutum   AACATACTATATCAAAAGAGATATCAGTCTGGTCTGATAAATAACTGATAAATTGCACGA
Hypericum majus      AACATACTATATCAAA--AGATTACAGTCTGGTCTGAAAAAGAACCGATTAATTGCACGA

SRM 3262             GAATAAAGATAGAGTCCCAGTCTACATGTCAATATCGACATAAAGGAAATTTATAGTAAG
Hypericum perforatum GAATAAAGATAGAGTCCCAGTCTACATGTCAATATCGACATAAAGGAAATTTATAGTAAG
Hypericum punctatum  GAATAAAGATAGAGTCCCAGTCTACATGTCAATATCGACATAAAGGAAATTTATAGTAAG
Hypericum maculatum  GAATAAAGATAGAGTCCCAGTCTACATGTCAATATCGACATAAAGGAAATTTATAGTAAG
Hypericum undulatum  GAATAAAGATAGAGTCCCAGTCTACATGTCAATATCGACATAAAGGAAATTTATAGTAAG
Hypericum hirsutum   GAATAAAGATAGAGTCCCAGTCTACATGTCAATATCGACATAAAGGAAATTTATAGTAAG
Hypericum majus      GAATAAAGATAGAGTCCCATTCTACATGTCAATATCGACACAAAGGAAATTTATAGTAAG

SRM 3262             AGGAAAATCCGTCGACTTTATAAATCGTGAGGGTTCAAGTCCCTCTATCCCCAGTCTCCT
Hypericum perforatum AGGAAAATCCGTCGACTTTATAAATCGTGAGGGTTCAAGTCCCTCTATCCCCAGTCTCCT
Hypericum punctatum  AGGAAAATCCGTCGACTTTATAAATCGTGAGGGTTCAAGTCCCTCTATCCCCAGTCTCCT
Hypericum maculatum  AGGAAAATCCGTCGACTTTATAAATCGTGAGGGTTCAAGTCCCTCTATCCCCAGTCTCCT
Hypericum undulatum  AGGAAAATCCGTCGACTTTATAAATCGKGAGGGTTCAAGTCCCTCTATCCCCAGTCTCCT
Hypericum hirsutum   AGGAAAATCCGTCGACTTTATAAATCGTGAGGGTTCAAGTCCCTCTATCCCCAGTCTCCT
Hypericum majus      AGGAAAATCCGTCGACTTTATAAATCGTGAGGGTTCAAGTCCCTCTATCCCCAGTTCCCT

SRM 3262             CGCCTTTT--TTTTTTGATCTGATTCTTTCTTTTCGTTAACGCTTGAATATTCATTATCTT
Hypericum perforatum CGCCTTTT--TTTTTTGATCTGATTCTTTCTTTTCGTTAACGCTTGAATATTCATTATCTT
Hypericum punctatum  CGCCTT---TTTTTTGATCTGATTCTTTCTTTTCGTTAACGCTTGAATATTCATTATCTT
Hypericum maculatum  CGCCTTT--TTTTTTGATCTGATTCTTTCTTTTCGTTAACGCTTGAATATTCATTATCTT
Hypericum undulatum  CGCCTTT-TTTTTTGATCTGATTCTTTCTTTTCGTTAACGCTTGAATATTCATTATCTT
Hypericum hirsutum   CGCCTTTAITTTTTTGATCTGATTCTTTCTTTTCGTTAACCCTTGAATATTCATTATCTT
Hypericum majus      CGCCT---TTTTTTGATCTGATTCTTTCTTTTCGTTAACGCTTTCAAATTCATTTTCTT

SRM 3262             TTTCATTGATTCGATTGTTTCACAAA
Hypericum perforatum TTTCATTGATTCGATTGTTTCACAAA
Hypericum punctatum  TTTCATTGATTCGATTGTTTCACAAA
Hypericum maculatum  TTTCATTGATTCGATTGTTTCACAAA
Hypericum undulatum  TTTCATTGATTCGATTGTTTCACAAA
Hypericum hirsutum   TTTCATTGATTCGATTGTTTCACAAA
Hypericum majus      TTTCATTGATTCGATTTTTTCACAAA
```

Figure 1. *trnL-trnF* DNA Aligned Matrix for *Hypericum perforatum L.* and Relatives. The results from the chloroplast region demonstrates that this region does distinguish this species from its relatives and does authenticate *Hypericum perforatum L.* The individual bases are represented as A = Adenine, T = Thymine, G = Guanine, C= Cystosine, and U = Uracil. Polymorphic bases are represented by standard IUPAC codes with R = A/G, W = A/T, M = A/c, Y = C/T, S = G/C, K = G/T, and N = missing data. The confidence estimate for the species identification of SRM 3262 as *Hypericum perforatum L.* is Most Confident (0).

Appendix C

rbcL DNA Aligned Matrix for *Hypericum perforatum L.* and Relatives

```
SRM 3262              ATTGACTTATTATACTCCTGACTATGAAACTAAAGATACTGATATCTTGGCAGCATTTCG
Hypericum perforatum  ATTGACTTATTATACTCCTGACTATGAAACTAAAGATACTGATATCTTGGCAGCATTTCG
Hypericum punctatum   ATTGACTTATTATACTCCTGACTATGAAACTAAAGATACTGATATCTTGGCAGCATTTCG
Hypericum undulatum   ATTGACTTATTATACTCCTGACTATGAAACCAAAGATACTGATATCTTGGCAGCATTTCG
Hypericum hirsutum    ATTGACTTATTATACTCCTGACTATGAAACCAAGGATACTGATATCTTGGCAGCATTTCG

SRM 3262              AGTAACTCCTCAACCTGGCGTTCCACCGGAGGAAGCAGGAGCAGCGGTAGCTGCGGAATC
Hypericum perforatum  AGTAACTCCTCAACCTGGCGTTCCACCGGAGGAAGCAGGAGCAGCGGTAGCTGCGGAATC
Hypericum punctatum   AGTAACTCCTCAACCTGGCGTTCCACCGGAGGAAGCAGGAGCAGCGGTAGCTGCGGAATC
Hypericum undulatum   AGTAACTCCTCAACCTGGCGTTCCACCGGAGGAAGCAGGAGCAGCGGTAGCTGCGGAATC
Hypericum hirsutum    AGTAACTCCTCAACCTGGCGTTCCACCGGAGGAAGCAGGAGCAGCGGTAGCTGCGGAATC

SRM 3262              TTCTACTGGTACCTGGACAACTGTTTGGACAGATGGACTGACCAGTCTTGATCGTTATAA
Hypericum perforatum  TTCTACTGGTACCTGGACAACTGTTTGGACAGATGGACTGACCAGTCTTGATCGTTATAA
Hypericum punctatum   TTCTACTGGTACCTGGACAACTGTTTGGACCGATGGACTGACCAGTCTTGATCGTTATAA
Hypericum undulatum   TTCTACTGGTACCTGGACAACTGTTTGGACAGATGGACTGACCAGTCTTGATCGTTATAA
Hypericum hirsutum    TTCTACTGGTACCTGGACAACTGTCTGGACAGATGGACTGACCAGTCTTGATCGTTATAA

SRM 3262              AGGACGATGCTACCACATTGAGCCTGTTCCTGGAGAGGAAAATCAATTTATTGCTTATGT
Hypericum perforatum  AGGACGATGCTACCACATTGAGCCTGTTCCTGGAGAGGAAAATCAATWTATTGCTTATGT
Hypericum punctatum   AGGACGATGCTACCACATTGAGCCTGTTCCTGGAGAGGAAAATCAATATATTGCTTATGT
Hypericum undulatum   AGGACGATGCTACCACATTGAGCCTGTTCCTGGAGAGGAAAATCAATATATTGCTTATGT
Hypericum hirsutum    AGGACGATGCTACCACATTGAGCCTGTTCCTGGAGAGGAAAATCAATATATTGCTTATGT

SRM 3262              AGCTTACCCCTTAGACCTTTTTGAGGAAGGTTCTGTTACTAACATGTTTACTTCCATTGT
Hypericum perforatum  AGCTTACCCCTTAGACCTTTTTGAGGAAGGTTCTGTTACTAACATGTTTACTTCCATTGT
Hypericum punctatum   AGCTTACCCCTTAGACCTTTTTGAGGAAGGTTCTGTTACGAACATGTTTACTTCCATTGT
Hypericum undulatum   AGCTTACCCCTTAGACCTTTTTGAGGAAGGTTCTGTTACTAACATGTTTACTTCCATTGT
Hypericum hirsutum    AGCTTACCCCTTAGACCTTTTTGAGGAAGGTTCTGTTACTAATATGTTTACTTCCATTGT

SRM 3262              AGGTAATGTATTTGGATTCAAAGCCCTGCGTGCTCTCCGGTTAGAGGATTTGCGAATCCC
Hypericum perforatum  AGGTAATGTATTTGGATTCAAAGCCCTGCGTGCTCTCCGGTTAGAGGATTTGCGAATCCC
Hypericum punctatum   AGGTAATGTATTTGGATTCAAAGCCCTGCGTGCTCTCCGGTTAGAGGATTTGCGAATCCC
Hypericum undulatum   AGGTAATGTATTTGGATTCAAAGCCCTGCGTGCTCTCCGGTTAGAGGATTTGCGAATCCC
Hypericum hirsutum    AGGTAATGTATTTGGATTCAAAGCCCTGCGTGCTCTCCGGTTAGAGGATTTGCGAATCCC

SRM 3262              TCCTGCTTATACTAAAACTTTCCAAGGTCCGCCCCACGGCATCCAAGTTGAAAGAGATAA
Hypericum perforatum  TCCTGCTTATACTAAAACTTTCCAAGGTCCGCCCCACGGCATCCAAGTTGAAAGAGATAA
Hypericum punctatum   CCCTGCTTATACTAAAACTTTCCAAGGTCCGCCCCACGGCATCCAAGTTGAAAGAGATAA
Hypericum undulatum   TCCTGCTTATACTAAAACTTTCCAAGGTCCGCCCCACGGCATCCAAGTTGAAAGAGATAA
Hypericum hirsutum    TCCTGCTTATACTAAAACTTTCCAAGGTCCACCCCACGGCATCCAAGTTGAAAGAGATAA

SRM 3262              ATTAAACAAGTATGGCCGTCCCCTATTAGGTTGTACAATTAAACCTAAATTGGGGTTATC
Hypericum perforatum  ATTAAACAAGTATGGCCGTCCCCTATTAGGTTGTACAATTAAACCTAAATTGGGGTTATC
Hypericum punctatum   ATTAAACAAGTATGGCCGTCCCCTATTAGGTTGTACAATTAAACCTAAATTGGGGTTATC
Hypericum undulatum   ATTAAACAAGTATGGCCGTCCCCTATTAGGTTGTACAATTAAACCTAAATTGGGGTTATC
Hypericum hirsutum    ATTAAACAAGTATGGCCGTCCCTTATTAGGTTGTACAATTAAACCTAAATTGGGGTTATC

SRM 3262              CGCTAAGAATT
Hypericum perforatum  CGCTAAGAATT
Hypericum punctatum   CGCTAAGAATT
Hypericum undulatum   CGCTAAGAATT
Hypericum hirsutum    CGCTAAGAATT
```

Figure 2. rbcL DNA Aligned Matrix for *Hypericum perforatum L.* and Relatives. The results from the chloroplast region demonstrates that this region does distinguish this species from its relatives and does authenticate *Hypericum perforatum L.* The four bases of DNA are represented as A = Adenine, T = Thymine, G = Guanine, and C = Cytosine. Polymorphic bases are represented by standard IUPAC codes with R = A/G, W = A/T, M = A/c, Y = C/T, S = G/C, K = G/T, and N = missing data. The confidence estimate for the species identification of SRM 3262 as *Hypericum perforatum L.* is Most Confident (0).

ITS DNA Aligned Matrix for *Hypericum perforatum L.* and Relatives

```
SRM 3262               CCTCCAAATGACCCGCGAACTAGTTATCCACAAGTCGGGGGTGTCGTGGGCTTCTTTCCC
Hypericum perforatum   CCTYCRAATGACCCGCGAACTAGTTATCCACAAGTCGGGGGTGTCGTGTGGGCTTCTGTCCC
Hypericum quadrangulum CCTCCAAATGACCCGCGAACTAGTTATCCACAAGTCGGGGGTGTCGTGTGGGCTTCTGTCCC
Hypericum maculatum    CCTCCAAATGACCCGCGAACTAGTTATCCACAAGTCGGGGGTGTCGTGTGGGCTTCTGTCCC
Hypericum undulatum    CCTCCAAATGACCCGCGAACTAGTTATCCACAAGTCGGGGGTGTCGTGTGGGCTTCTGTCCC
Hypericum hirsutum     CCTCCAAATGACCCGCGAACCAGTTATCCACAAGT-GGGGGTGTCG--GGCTT-CGTCCC
Hypericum majus        CTTGCAAAAGACCCGTGAACTAGTTATCAACATGTGGGGAGTGTTGGGAGATA-TCCCTT

SRM 3262               GGCGCCCCCCGGGGCGGTGGTGGCCAGGCGCGCCAAGYTYTTGGCACGGYTGGCCCATCWC
Hypericum perforatum   GGCGCCCCCCTGSCGGTGGTGGCCAGGCYGCCAAGCTYTTGGGWCGGCTGGCYCATCAC
Hypericum quadrangulum GGCGCCCCCCTGCCGGTGGTGGCCAGGCGCGCCAAGCTCTTGGCACGGCTGGCCCATCAC
Hypericum maculatum    GGCGCCCCCCTGCCGGTGGTGGCCAGGCGCGCCAAGCTCTTGGCACGGCTGGCCCATCAC
Hypericum undulatum    GGCGCCCCCCTGCCGGTGGTGGCCAGGCGCGCCAAGCTTTTGGCACGGCTGGCCCATCAC
Hypericum hirsutum     GGCGCCCCCGTTTGGGTGGTGGTCAGGCGCGCCAAGCTCTTGGCACGGCTGTTCATCAC
Hypericum majus        AGCTCCTTTGTGCCAGTGGTGGGTAATTGTGTCGTTATACCGGCATGATTGACCAACATC

SRM 3262               CCGCCCAACAAACAAACCCCGGCGCGGCACGCGCCAAGGAACTTTTGCATCATAAGAAGY
Hypericum perforatum   CTGCCCAACAAACAAACCCCGGCGCGGCAYGCGCCAAGGAACTTTTGCATCATAAGAAGY
Hypericum quadrangulum CTGCCCAACAAACAAACCCCGGCGCGGCACGCGCCAAGGAACTTTTGCATCATAAGAAGT
Hypericum maculatum    CTGCCCAACAAACAAACCCCGGCGCGGCACGCGCCAAGGAACTTTTGCATCATAAGAAGT
Hypericum undulatum    CTGCCCAACAAACAAACCCCGGCGCGGCACGCGCCAAGGAACTTTTGCATCATAAGAAGT
Hypericum hirsutum     CTGCCCAACAAACAAACCCCGGCGCGGCACGCGCCAAGGAACTTTTGCAATATAAGAACG
Hypericum majus        TGGACAAACTAACCAACCCCGGCGCGGCATGCGCCAAGGAACTT---CGCAATAAATGTG

SRM 3262               GTAAKGCTCCCGGCTGTG-CCGGAAATCGGACAACACG-----GTYGGGGGCYTCCTTCT
Hypericum perforatum   GTAAKGCTCCCGGCTGTG-CCGGAAATCGGACAACACG-----GTYGGGGGCYTCCTTCT
Hypericum quadrangulum GTAAGGCTCCCGGCTGTG-CCGGAAATCGGACAACACG-----GTCGGGGGCTTCCTTCT
Hypericum maculatum    GTAAGGCTCCCGGCTGTG-CCGGAAATCGGACAACACG-----GTCGGGGGCTTCCTTCT
Hypericum undulatum    GTAAGGCTCCCGGCTGTG-CYGGAAATCGGACAACACG-----GTCGGGGGCTTCCTTCT
Hypericum hirsutum     ATCACGCTCCCGGCCGTG-CCGGAAATCGGACAACGCG-----GTCGGGGGCTTCCTTCT
Hypericum majus        GAAACGCTACCGGTCGTT-CCGAAAATCGGATAAGCGGCCGTGTTGTGGACCACTT--

SRM 3262               GTTCATAACAATAACGACTCTCGGCAACGGATATCTAGGCTCTTGCATCGATGAAGAACG
Hypericum perforatum   GTTCATAACAATAACGACTCTCGGCAACGGATATCTAGGCTCTTGCATCGATGAAGAACG
Hypericum quadrangulum GTTCATAACAATAACGACTCTCGGCAACGGATATCTAGGCTCTTGCATCGATGAAGAACG
Hypericum maculatum    GTTCATAACAATAACGACTCTCGGCAACGGATATCTAGGCTCTTGCATCGATGAAGAACG
Hypericum undulatum    GTTCATAACAATAACGACTCTCGGCAACGGATATCTAGGCTCTTGCATCGATGAAGAACG
Hypericum hirsutum     ATTCATAACAATAACGACTCTCGGCAACGGATATCTAGGCTCTTGCATCGATGAAGAACG
Hypericum majus        -TTCATTACAA-AACGACTCTCGGCAACGGATATCTAGGCTCTTGCATCGATGAAGAACG

SRM 3262               TAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAAC
Hypericum perforatum   TAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAAC
Hypericum quadrangulum TAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAAC
Hypericum maculatum    TAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAAC
Hypericum undulatum    TAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAAC
Hypericum hirsutum     TAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAAC
Hypericum majus        TAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAAC

SRM 3262               GCAAGTTGCGCCCGAAGCCTTCTGGCCGAGGGCACGCCTGCCTGGGTGTCACACATCGTC
Hypericum perforatum   GCAAGTTGCGCCCGAAGCCTTCTGGCCGAGGGCACGCCTGCCTGGGTGTCACACATCGTC
Hypericum quadrangulum GCAAGTTGCGCCCGAAGCCTTCTGGCCGAGGGCACGCCTGCCTGGGTGTCACACATCGTC
Hypericum maculatum    GCAAGTTGCGCCCGAAGCCTTCTGGCCGAGGGCACGCCTGCCTGGGTGTCACACATCGTC
Hypericum undulatum    GCAAGTTGCGCCCGAAGCCTTCTGGCCGAGGGCACGCCTGCCTGGGTGTCACACATCGTC
Hypericum hirsutum     GCAAGTTGCGCCCGAAGCCTTCTGGCCGAGGGCACGCCTGCCTGGGTGTCACACATCGTC
Hypericum majus        GCAAGTTGCGCCCGAAGCCTCTTGGCCAAGGGCACGTCTGCCTGGGTGTCACACATCGTC

SRM 3262               GCCCCCCAAAATCCCGATATCTYGCAAGAGACAATYGGGAATAGGATGGGCGGAAAATGG
Hypericum perforatum   GCCCCCCAAAATCYCGATATCTYGCAAGASACAATCGGGAATAGGATGGGCGGAAAATGG
Hypericum quadrangulum GCCCCCCAAAATCCCGATATCTCGCAAGACACAATCGGGAATAGGATGGGCGGAAAATGG
Hypericum maculatum    GCCCCCCAAAATCCCGATATCTCGCAAGACACAATCGGGAATAGGATGGGCGGAAAATGG
Hypericum undulatum    GCCCCCCAAAATCCCGATATCTCGCAAGAGACAATCGGGAATAGGATGGGCGGAAAATGG
Hypericum hirsutum     GCCCCCCAAAAT-CCGATCTCTCGCAAGGACGATCGGGAAGAGGATGGGCGGAAAATGG
Hypericum majus        -CCGACCGAAACAAATACTCCTCGTAGG---TGTTGGTAAGAGGATGGGCGGATAATGG

SRM 3262               TCTCCCGTGCGCTCCCGTTCGCGGTTGGCCCAAAAATGAGTTCCTGGC-AAAGCAAAGCC
Hypericum perforatum   TCTCCCGTGCGCTCCCGTTCGCGGTTGGCCCAAAAATGAGTTCYTGGC-AAAGCAAAGCC
Hypericum quadrangulum TCTCCCGTGCGCTCCCGTTCGCGGTTGGCCYAAAAATGAGTTCCTGGC-AAAGCAAAGCC
Hypericum maculatum    TCTCCCGTGCGCTCCCGTTCGCGGTTGGCCYAAAAATGAGTTCCTGGC-AAAGCAAAGCC
Hypericum undulatum    TCTCCCGTGCGCTCCCGTTCGCGGTTGGCCCAAAAATGAGTTCCTGGC-AAAGCAAAGCC
Hypericum hirsutum     TCTCCCGTGCGCTCCCGTTCGCGGTTGGCCCAAAAATGAGTTCCTGGC-AAAGCAAAGCC
Hypericum majus        TCTCCCGTGCGCGGTTGCTCGCGGTTGGCCTAAAAATAAGTTCCTGGTGATAGC-AAGCC
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SRM 3262                 ACGACCAGCGGTGGTTGTAAGACCCTCGGTACAAGTCGTGAG-CCTTGCATTGCTCGTAG
Hypericum perforatum     ACGACCAGCGGTGGTTGTAAGACCCTCGGTACAAGTCGTGAG-CCTTGCATTGCTYGTAG
Hypericum quadrangulum   ACGACCAGCGGTGGTTGTAAGACCCTCGGTACAAGTCGTGAG-CCTTGCATTGCTCGTAG
Hypericum maculatum      ACGACCAGCGGTGGTTGTAAGACCCTCGGTACAAGTCGTGAG-CCTTGCATTGCTCGTAG
Hypericum undulatum      ACGACCAGCGGTGGTTGTAAGACCCTCGGTACAAGTCGTGAG-CCTTGCATTGCGCGTAG
Hypericum hirsutum       ACGACCAGCGGTGGTTGTAAGACCCTCGGTACAAGTCGTGAG-CCTTGCGTTGCGAATAC
Hypericum majus          ATGACCAGCGGTGGTTGAAAGACCCTCGATGGGTGTCGTGAGAACTTGCATCGCATGTTG

SRM 3262                 GGACATGTTGACCCTGAACGTGATCGAGTAACATCGA-ACACTCACAA
Hypericum perforatum     GGACATGTTGACCCTGAACGTGATCGAGTAACATCGA-ACACTCACAA
Hypericum quadrangulum   GGACATGTTGACCCTGAACGTGATCGAGTAACATCGGTACACTCACAA
Hypericum maculatum      GGACATGTTGACCCTGAACGTGATCGAGTAACATCGGTACACTCACAA
Hypericum undulatum      GGACATGTTGACCCTGAACGTGATCGAGTAACATCGGTACACTCACAA
Hypericum hirsutum       GGACATGTTGACCCTGAACGTGATCGAGTAACTTCGA-ACGCTCACAA
Hypericum majus          GGAGATTCTGACCTTGATCGTGTTTAAGCA-TATCAA-ACACTCATGA
```

Figure 3. ITS DNA Aligned Matrix for *Hypericum perforatum L.* and Relatives. The results from the nuclear ribosomal gene region demonstrates that this region does distinguish this species from its relatives and is sufficient for identification of *Hypericum perforatum L.* The four bases of DNA are represented as A = Adenine, T = Thymine, G = Guanine, and C = Cytosine. Polymorphic bases are represented by standard IUPAC codes with R = A/G, W = A/T, M = A/c, Y = C/T, S = G/C, K = G/T, and N = missing data. The confidence estimate for the species identification of SRM 3262 as *Hypericum perforatum L.* is Most Confident (0).
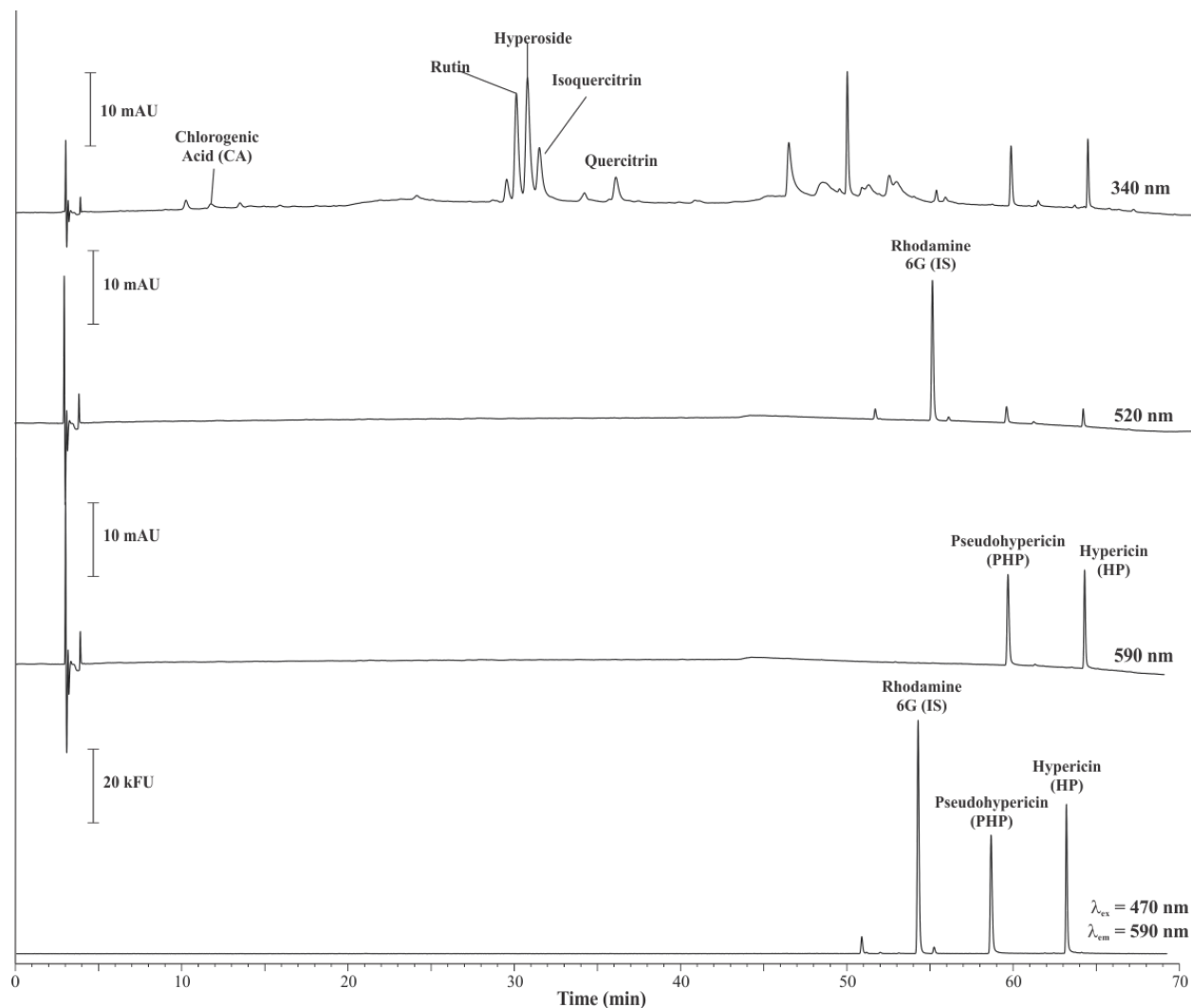
Typical Chromatograms

Figure 4. Chromatograms showing separation and detection of chlorogenic acid, flavonoids, and naphthodianthrones in SRM 3262 using LC with absorbance and fluorescence detection. A C18 column was held at 40 °C and the separation was performed using a gradient consisting of (A) aqueous 0.5 % triethylamine (volume fraction) adjusted to pH 4.5 with acetic acid and (B) acetonitrile.