National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 3248

Ginkgo-Containing Tablets

This Standard Reference Material (SRM) is intended primarily for use in validating analytical methods for the determination of flavonoids, terpene lactones, and toxic elements in ginkgo-containing tablets and similar matrices. This SRM can also be used for quality assurance when assigning values to in-house control materials. SRM 3248 is part of a suite of ginkgo dietary supplement SRMs that have been developed to cover a range of natural matrices and analyte levels. A unit of SRM 3248 consists of five bottles, each containing approximately 1 g of ground tablets.

The development of SRM 3248 was a collaboration among the National Institute of Standards and Technology (NIST); the National Institutes of Health (NIH), Office of Dietary Supplements (ODS); and the Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER).

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 [1]. The certified concentration values of selected flavonoids, terpene lactones, and lead are provided in Tables 1 and 2. Values were derived from the combination of results provided by NIST and collaborating laboratories. The certified values in this material are the equally weighted means of the individual sets of NIST results and the means of the combined sets of measurements made by collaborating laboratories; the associated uncertainties are expanded uncertainties at the 95 % level of confidence [2,3]. Values are reported on a dry-mass basis in mass fraction units [4].

Reference Values: 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. Reference concentration values for additional terpene lactones are provided in Table 3. Reference values for arsenic, cadmium, and mercury are provided in Table 4.

Expiration of Certification: The certification of **SRM 3248** is valid, within the measurement uncertainties specified, until **30 October 2019**, 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 L.C. Sander, K.E. Sharpless, and S.A. Wise of the NIST Chemical Sciences Division.

Support for the development of SRM 3248 was provided in part by the NIH Office of Dietary Supplements (ODS) and the FDA Center for Drug Evaluation and Research (CDER). Technical consultation from these agencies was provided by J. Betz (NIH ODS) and A. NguyenPho (FDA CDER).

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Acquisition and preparation of the material was coordinated by A. NguyenPho of FDA CDER and K. E. Sharpless.

Analytical measurements at NIST were performed by S.B. Howerton, S.E. Long, K.E. Murphy, B.J. Porter, K. Putzbach, M.S. Rearick, C.A. Rimmer, L.J. Wood, and R.L. Zeisler of the NIST Chemical Sciences Division and D. Hancock of the NIST Biochemical Science Division. Analyses for value assignment were also performed by C. Scriver and L. Yang of the National Research Council Canada (NRCC; Ottawa, ON); M Sanders at NSF International (Ann Arbor, MI), C. Nelson at Eurofins (Petaluma, CA), and B. Schaneberg at ChromaDex, Inc. (Boulder, CO). Data from an AOAC collaborative study for flavonoids in SRM 3248 were also included in value assignment; the directors for this study were D. Gray (Midwest Research Institute; Kansas City, MO), K. LeVanseler and M. Pan (NSF International, Ann Arbor, MI), and E. Waysek (Caravan Products Company: Totawa, NJ). Thin layer chromatographic analysis was provided by A. Blatter and E. Reich (CAMAG, Muttenz, Switzerland).

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

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

NOTICE AND WARNING TO USERS

For laboratory use only. 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 its unopened bottle, until required for use.

Use: Prior to removal of a test portion for analysis, the contents of a bottle of material should be mixed thoroughly. For certified values to be valid, test portions equal to or greater than 0.5 g for flavonoids, 1 g for terpene lactones, and 0.75 g for lead should be used. 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.

PREPARATION AND ANALYSIS[1]

Material Acquisition and Preparation: Approximately 7.4 kg of ginkgo tablets were ground at room temperature in a Teflon disk mill containing a concentric Teflon ring and a Teflon puck, and sieved to 180 µm (80 mesh). The sieved material was transferred to ChromaDex, Inc. (Santa Ana, CA) where it was blended and then bottled under nitrogen in amber high-density polyethylene bottles with polypropylene screw caps. After bottling, the material was irradiated by ^{60}Co to an absorbed dose of 12.9 kGy to 15.7 kGy.

Analytical Approach for Determination of Flavonoids

Value assignment of the concentrations of flavonoids in SRM 3248 was based on the combination of measurements from different analytical methods at NIST, at two collaborating laboratories, and in an interlaboratory comparison using a single analytical method. A total of five sets of measurements were used for the value assignment of the concentrations of flavonoids. NIST provided measurements by using a combination of two sample extraction procedures and three liquid chromatography (LC) methods with different detection, i.e., ultraviolet absorbance spectrometry (UV) and mass spectrometry (MS) as described below. Results for flavonoids were provided by two collaborating laboratories (NSF International and ChromaDex) and participants in an AOAC collaborative study. All collaborating laboratories' results were based on LC/UV. Two collaborating laboratories analyzed a minimum of six subsamples, one from each of six bottles or two from each of three bottles, and one laboratory analyzed one subsample from each of three bottles of SRM 3248.

[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.

NIST Analyses for Flavonoids

Flavonoid aglycones were measured by using combinations of two sample preparation methods and two LC methods with UV and MS detection. Four independently prepared calibrants were used for each of the methods. Calibrants were prepared gravimetrically, at levels intended to approximate levels of the flavonoid aglycones in the extracts of the SRMs. A single internal standard solution was used for calibrants and samples. Calculations are based on average response factors for the calibrants (typically duplicate analysis of four calibrant solutions, n = 8). The purity of the standards was determined and was used in the calculation of the results. In addition, the water content of quercetin was also corrected since this standard is hydrated in solid form.

Soxhlet Extraction: Twelve 0.5 g portions of the SRM were weighed into glass-fritted Soxhlet thimbles each containing an approximately 1 cm layer of diatomaceous earth (Hydromatrix, Isco, Lincoln, NE). After stirring the sample, a measured mass of internal standard solution (hesperitin) was transferred to the Soxhlet thimble. The samples were extracted with approximately 200 mL methanol for 24 h. Samples prepared by this approach were hydrolyzed as described below and then analyzed by LC/UV.

Pressurized Fluid Extraction: Twelve 0.5 g portions of the SRM were placed into stainless steel extraction vessels fitted with a cellulose disk to prevent obstruction of the fritted stainless steel disk in the end cap. Each vessel was filled to approximately one half capacity with Hydromatrix. After stirring the sample, a measured mass of internal standard solution (hesperetin) was added, and an additional layer of Hydromatrix material was added to fill the vessel completely. The extraction vessels were extracted three times each with three static holds of 5 min per extraction cycle. A nominal pressure of 13.8 MPa (2000 psi) and a temperature of 100 ºC were maintained. Samples prepared by this approach were hydrolyzed as described below and then analyzed by LC/MS.

Hydrolysis: After extraction, 30 g of the extract was refluxed with approximately 10 g of 25 % hydrochloric acid solution (mass fraction) to cleave the sugar residues from the various glycosides to produce the aglycones.

LC /UV Absorbance Detection: A C_{18} column was used with a binary gradient LC method (mobile phase of water and acetonitrile, both containing acetic acid) for the LC/UV determination. The aglycones were detected at 370 nm, and the internal standard was detected at 287 nm. A typical separation is provided in Appendix A.

LC/MS Detection: A C_{18} column was used with an isocratic mobile phase (water/acetonitrile/acetic acid/trifluoroacetic acid) for the LC/MS determination. Positive electrospray mode was used for the determination of the flavonoid aglycones. Quantification of the aglycones was based on selected ion monitoring at *m/z* 303 for quercetin and hesperetin, *m/z* 317 for isorhamnetin, and *m/z* 287 for kaempferol. Hesperitin used as the internal standard for LC/MS measurements. A typical separation is provided in Appendix A.

ANALYTICAL APPROACH FOR DETERMINATION OF TERPENE LACTONES

Value assignment of the concentrations of the terpene lactones in SRM 3248 was based on the combination of measurements from two different analytical methods at NIST and at one collaborating laboratory. A total of three sets of measurements was used for the value assignment of the concentrations of terpene lactones. NIST provided measurements by using two different methods for sample extraction and two different LC methods with MS as described below. Results for terpene lactones were also provided by Eurofins (Petaluma, CA), who analyzed samples using LC with evaporative light scattering detection (ELSD). NIST analyzed single test portions from each of ten bottles or duplicate test portions from each of six bottles, and Eurofins analyzed single test portions from each of five bottles.

NIST Analyses for Terpene Lactones

Terpene lactones were measured by using combinations of two sample preparation methods and three LC/MS methods. Five independently prepared five-component calibrants were used for Methods 1 and 3; six were used for Method 2. Calibration solutions were prepared gravimetrically, at levels intended to approximate the levels of the terpene lactones in the extracts of the SRM. A single internal standard solution was used for the calibrants and samples. Calculations are based on average response factors for typically three injections of each of the calibration solutions.

Soxhlet Extraction: Ten 1 g portions of the SRM were weighed into glass-fritted Soxhlet thimbles each containing an approximately 2.5 cm layer of diatomaceous earth (Hydromatrix, Isco, Lincoln, NE). After stirring the sample, a measured mass of internal standard solution (limonin) was transferred to the Soxhlet thimble. The samples were extracted with approximately 200 mL methanol for 22 h. Samples prepared by this approach were analyzed by LC/MS Method 1 and Method 3.

Sonication Extraction: Twelve 1 g portions of the SRM taken from six bottles were placed in 50 mL polyethylene centrifuge tubes, followed by the addition of a measured mass of internal standard solution (hesperitin). Approximately 10 mL of methanol was added to the tubes, and the tubes were capped. The solid matter was suspended by shaking, and the tubes were placed in an ultrasonic bath for 30 min. At the completion of the sonication extraction, the samples were centrifuged, and the methanol was removed and replaced with fresh methanol. The extraction was performed five times, and the five portions of methanol were combined and analyzed by LC/MS Method 2.

LC/MS Method 1: A C_{12} column was used with a mobile phase gradient (water/methanol/acetic acid) for the LC/MS Method 1 determination. Positive ion electrospray mass spectrometry was used for detection of the terpene lactones. Quantification was based on monitoring ions *m/z* 344 for bilobalide, *m/z* 426 for ginkgolide A, *m/z* 442 for ginkgolides J and B, *m/z* 458 for ginkgolide C, and *m/z* 488 for limonin. Limonin was used as the internal standard. A typical separation is provided in Appendix B.

LC/MS Method 2: A C_{18} column was used with a mobile phase gradient (water/acetonitrile/acetic acid) for the LC/MS Method 2 determination. Positive ion electrospray mass spectrometry was used for detection of the terpene lactones. Quantification was based on monitoring ions *m/z* 327 bilobalide, *m/z* 409 for ginkgolide A, *m/z* 425 for ginkgolides J and B, *m/z* 441 for ginkgolide C, and *m/z* 303 for hesperitin. Hesperitin was used as the internal standard. A typical separation is provided in Appendix B.

LC/MS Method 3: A C_{18} column was used with a mobile phase gradient (water/acetonitrile/acetic acid) for the LC/MS Method 3 determination. Negative ion electrospray mass spectrometry was used for detection of the terpene lactones. Quantification was based on monitoring ions *m/z* 325 for bilobalide, *m/z* 467 for ginkgolide A, *m/z* 423 for ginkgolides J and B, *m/z* 483 for ginkgolide J, *m/z* 439 for ginkgolide C, and *m/z* 301 for hesperitin. Hesperitin was used as the internal standard. A typical separation is provided in Appendix B.

ANALYTICAL APPROACH FOR DETERMINATION OF ELEMENTS

The elements of primary interest for SRM 3248 were the potentially toxic contaminants arsenic, cadmium, lead, and mercury. Value assignment of the concentrations of toxic elements in SRM 3248 was based on the combination of measurements at NIST using a single analytical method and results from a collaborating laboratory (NRCC) when available. At NIST instrumental neutron activation analysis (INAA) was used for the determination of arsenic, isotope dilution inductively coupled plasma mass spectrometry (ID ICP-MS) was used for determination of cadmium and lead, and cold vapor (CV) ID ICP-MS was used for determination of mercury. For all NIST measurements, botanical-matrix SRMs with certified values for the elements of interest were analyzed concurrently as control samples. NRCC used ID ICP-MS for the determination of cadmium and lead and hydride generation graphite furnace atomic absorption spectrometry (HG GFAAS) for the determination of arsenic. NRCC analyzed six subsamples of SRM 3248.

NIST Analyses for Elements

Arsenic was determined by using INAA. Individual disks were formed from 100 mg test portions of the SRM using a stainless steel die and hydraulic press. Standards were prepared by transferring a weighed portion of a solution containing a known amount of arsenic onto filter papers. Disks were formed from the dried filter papers. Samples, standards, and controls were packaged individually in clean polyethylene bags, placed together in a polyethylene irradiation container, and exposed to a neutron fluence rate of 1×10^{14} $cm^{-2} \cdot s^{-1}$ for a total of 4 h. Decay times were approximately 4 d to 4.3 d. Gamma rays were collected using an intrinsic germanium detector with a relative efficiency of 35 % and a resolution of 1.75 keV (full-width at half maximum peak height for the 1333 keV line from ^{60}Co). Quantification was based on comparison with standards using the 559-keV and 658-keV lines from ^{76}As.

For cadmium and lead determinations, a single 0.75 g portion was taken from each of six bottles of the SRM. Isotopically enriched ^{111}Cd and ^{206}Pb spike solutions were added to the samples prior to digestion in PFA Teflon vessels with nitric and hydrofluoric acids using a high-throughput microwave system. The microwave digests were transferred to PFA Teflon beakers and heated to evaporate the acids, after which residue was redissolved in 2 % nitric acid. The analyte concentration of the spike solutions added to the samples was determined by reverse ID ICP-MS using primary Pb and Cd standards prepared from high-purity metals. Measurements were made by using quadrupole ICP-MS [5].

Because of potential interferences at the Cd masses, a matrix separation was performed on a single sample of SRM 3248 to estimate the uncertainty due to interference [6]. Samples were evaporated to dryness with concentrated hydrochloric acid to convert residual salts from the nitrate to the chloride form. Samples were redissolved in a mixture of hydrochloric and hydrofluoric acids, separated using anion exchange chromatography, evaporated, and redissolved

in nitric acid. There was a 3 % difference in determined Cd concentration between the separated and unseparated samples of SRM 3248.

For mercury determinations, a single 0.25 g portion was taken from each of six bottles of the SRM. Isotopically enriched ^{201}Hg was added to the samples prior to digestion in quartz vessels with nitric acid in a high-pressure microwave system. Following digestion, samples were diluted and allowed to degas overnight at 4 °C. Measurements were made by using cold-vapor mercury generation (using tin (II) chloride reductant) coupled with ICP-MS [7].

NIST Determination of Moisture

Moisture content of SRM 3248 was determined by (1) freeze-drying to constant mass over 14 days; (2) drying over magnesium perchlorate in a desiccator at room temperature for 12 days; and (3) drying for 2 h in a forced-air oven at 80 °C. Unweighted results obtained using all three techniques were averaged to determine a conversion factor of 0.9522 gram dry mass per gram as-received mass, which was used to convert data from an as-received to a dry-mass basis; NIST arsenic data were moisture-corrected by the analyst using a factor of 0.9556 as determined by drying two 1 g samples over magnesium perchlorate for 20 days. A variability-in-moisture component is included in the uncertainties of both the certified and reference values, reported on a dry-mass basis, that are provided in this certificate.

Homogeneity Assessment

The homogeneity of flavonoids and terpene lactones in SRM 3248 was assessed at NIST by using the methods described above. An analysis of variance did not show inhomogeneity for flavonoids and terpene lactones for the sample sizes employed. Other measurands were treated as though they were homogeneously distributed, although homogeneity was not assessed.

Assignment Values

The equally weighted means from each set of data were used to calculate the assigned values. Each NIST mean was averaged with the grand mean of data provided by collaborating laboratories.

Certified Mass Fraction Values for Flavonoid Aglycones and Selected Terpene Lactones: Each certified concentration value, expressed as a mass fraction on a dry-mass basis, is an equally weighted mean of results from analytical methods carried out at NIST and at collaborating laboratories. The uncertainty in the certified value, calculated according to the method described in the ISO/JCGM Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory, within-laboratory, and drying components of uncertainty. The coverage factor (k) is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and approximately 95 % confidence for each analyte. The measurands are the total mass fraction listed and the value listed is metrologically traceable to the SI unit of mass, expressed as milligrams per gram on a dry-mass basis.

Table 1. Certified Mass Fraction Values for Flavonoid Aglycones and Selected Terpene Lactones in SRM 3248

	Mass Fraction (mg/g)
Quercetin[a,b,c,d,e]	7.56 ± 0.40
Kaempferol[a,b,c,d]	7.19 ± 0.70
Isorhamnetin[a,b,c,d,e]	1.90 ± 0.22
Total Aglycones[a,b,c,e]	16.6 ± 1.2
Ginkgolide B[f,g]	1.12 ± 0.20
Ginkgolide C[f,g]	2.36 ± 0.42
Total Terpene Lactones[f,g]	11.8 ± 1.4

[a] NIST LC/UV
[b] NIST LC/MS
[c] ChromaDex LC/UV
[d] NSF International LC/UV
[e] AOAC collaborative study
[f] Two NIST LC/MS methods; because of variability in the response factors, LC/MS Method 3 was not used for value assignment of ginkgolides B and C and total terpene lactones
[g] Eurofins LC/ELSD

Certified Mass Fraction Value for Lead: The certified concentration value, expressed as a mass fraction on a dry-mass basis, is an equally weighted mean of the results from NIST and NRCC. The uncertainty in the certified value, calculated according to the method described in the ISO/JCGM Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory, within-laboratory, and drying components of uncertainty. The coverage factor (k) is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and approximately 95 % confidence for lead. The measurand is the total mass fraction listed for lead and the value listed is metrologically traceable to the SI unit of mass, expressed as micrograms per gram on a dry-mass basis.

Table 2. Certified Mass Fraction Value for Lead in SRM 3248

	Mass Fraction (µg/g)
Lead[a,b]	0.7753 ± 0.0089

[a] NIST ID ICP-MS
[b] NRCC ID ICP-MS

Reference Mass Fraction Values for Selected Terpene Lactones: Each reference concentration value, expressed as a mass fraction on a dry-mass basis, is an equally weighted mean of the results from three or four analytical methods carried out at NIST and at collaborating laboratories. The uncertainty in the reference value, calculated according to the method described in the ISO/JCGM Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory, within-laboratory, and drying components of uncertainty. The coverage factor (k) is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and approximately 95 % confidence for each analyte. The measurands are the mass fraction listed as determined by the method indicted and the value listed is metrologically traceable to the SI unit of mass, expressed as micrograms per gram on a dry-mass basis.

Table 3. Reference Mass Fraction Values for Selected Terpene Lactones in SRM 3248

	Mass Fraction (mg/g)
Ginkgolide A[a,b,c,d]	2.42 ± 0.63
Ginkgolide J[a,b,c,d]	0.81 ± 0.36
Bilobalide[a,b,c,d]	5.7 ± 1.2

[a] NIST LC/MS Method 1
[b] NIST LC/MS Method 2
[c] NIST LC/MS Method 3
[d] Eurofins LC/ELSD

Reference Mass Fraction Values for Arsenic, Cadmium, and Mercury: Each reference value, expressed as a mass fraction on a dry-mass basis, is an equally weighted mean of NIST results. The uncertainty in the reference value, calculated according to the method described in the ISO/JCGM Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory, within-laboratory, and drying components of uncertainty. The uncertainty for cadmium contains an additional component of uncertainty representing the difference in cadmium results for separated and unseparated samples. The coverage factor (k) is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and approximately 95 % confidence for each analyte. The measurands are the mass fraction listed as determined by the method indicted and the value listed is metrologically traceable to the SI unit of mass, expressed as nanograms per gram on a dry-mass basis.

Table 4. Reference Mass Fraction Values for Arsenic, Cadmium, and Mercury in SRM 3248

	Mass Fraction (ng/g)
Arsenic[a]	56.5 ± 4.3
Cadmium[b]	1.56 ± 0.19
Mercury[c]	0.271 ± 0.034

[a] NIST INAA
[b] NIST ID ICP-MS
[c] NIST CV ID ICP-MS

Supplemental Information

In addition to the analyses described above, further characterization of SRM 3248 was provided using thin layer chromatography (TLC). The experimental procedures and the results are provided in Appendices C1 through C3. These results are provided only as supplemental information to assist in characterizing SRM 3248 and are not intended for use in identifying dietary supplement tablets containing *Ginkgo biloba*.

REFERENCES

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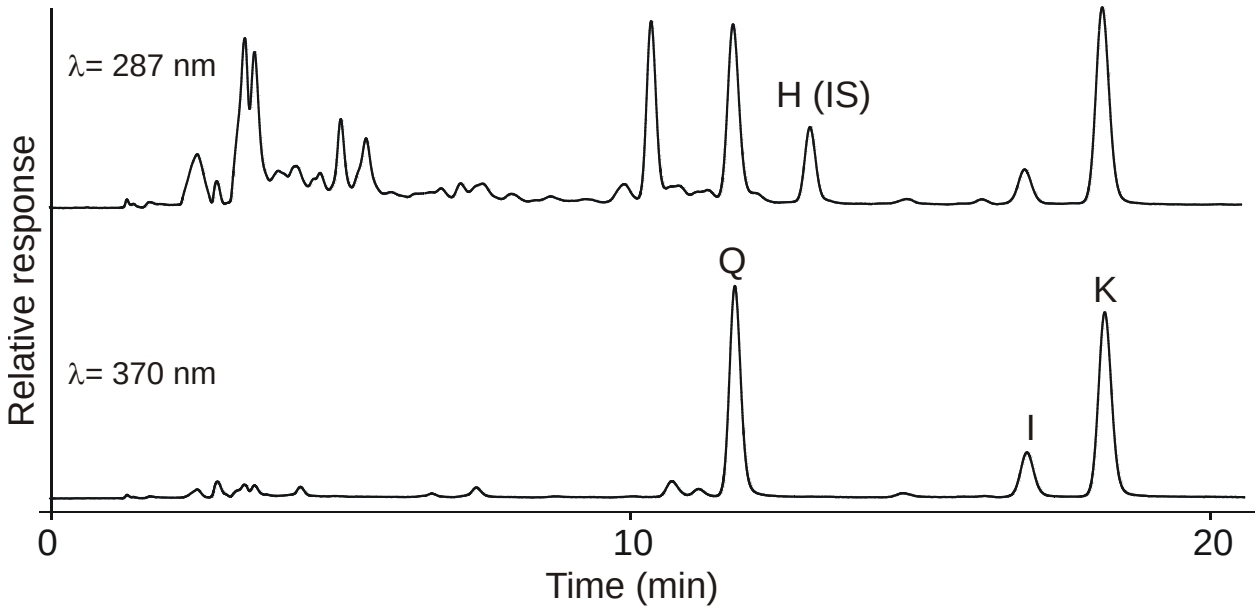
Certificate Revision History: 04 September 2014 (Extension of certification period; editorial changes); 27 July 2007 (Original certificate date)

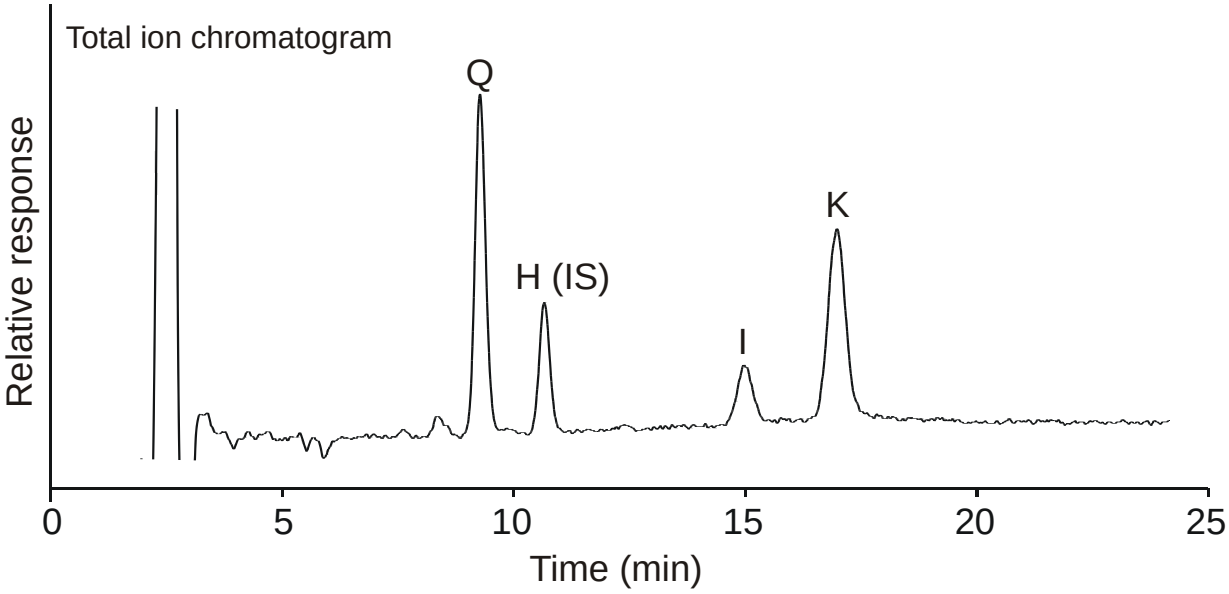
Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet http://www.nist.gov/srm.

Typical chromatograms from the analysis of flavonoid aglycones in SRM 3248 by using: (a) LC/UV and (b) LC/MS. For LC/UV, a binary gradient LC method with a water/acetonitrile (both containing acetic acid) mobile phase was used. A 0.46 cm x 25 cm Xterra (Waters, Milford, MA) C_{18} column was used with a SecurityGuard precolumn (C_{18} cartridge) and an in-line filter (0.5µm). A new precolumn and filter were used for each set of measurements. Column temperature was controlled at 25.0 ºC ± 2 ºC with a circulating-water column jacket and water bath. For LC/MS, a 0.46 cm x 25 cm Xterra C_{18} column was used at 25.0 ºC ± 2 ºC with a SecurityGuard precolumn (C_{18} cartridge) and an in-line filter with an isocratic mobile phase (water/acetonitrile/acetic acid/trifluoroacetic acid) at 1.0 mL/min. Positive electrospray mode was used for the determination of the flavonoid aglycones. Quantification of the the aglycones was based on selected ion monitoring at *m/z* 303 (quercetin, hesperetin), 317 (isorhamnetin), and 287 (kaempferol). Components are identified as follows: hesperitin (H; the internal standard), quercetin (Q), kaempferol (K), isorhamnetin (I).

(a) LC/UV method 1



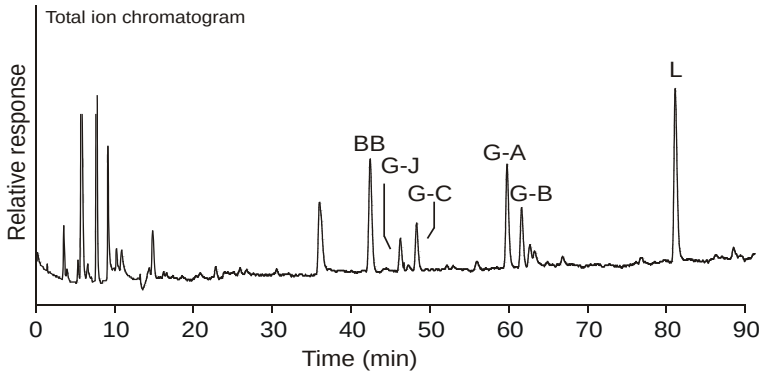
(b) LC/MS method 2



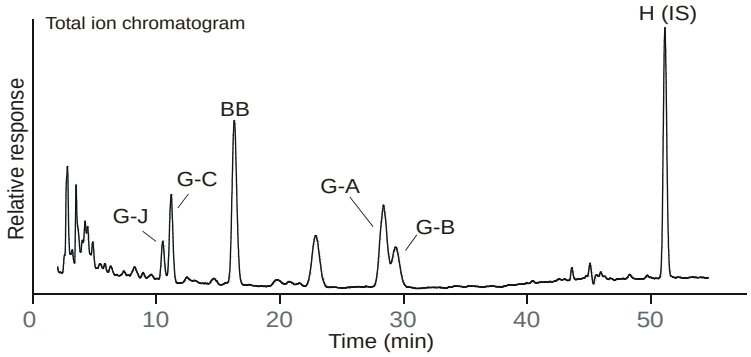
Appendix B

Typical chromatograms from the analysis of ginkgolides and bilobalide in SRM 3248 by using: (a) LC/MS method 1, (b) LC/MS method 2, and (c) LC/MS method 3. For LC/MS Method 1, a 250 mm x 4.6 mm Synergi-Max RP column (Phenomenex, Madrid, CA) and Synergi-Max RP guard column (Phenomenex) were held at 25 °C ± 1 °C with a column oven. A mobile phase gradient (water/methanol/acetic acid) and a flow rate of 0.75 mL/min were used. Positive ion electrospray mass spectrometry was used for detection of the terpene lactones. Quantification was based on monitoring ions (*m/z*) at 344 (bilobalide), 426 (ginkgolide A), 442 (ginkgolides J and B), 458 (ginkgolide C) and 488 (limonin); limonin was used as the internal standard. For LC/MS Method 2, a 250 mm x 4.6 mm Xterra C_{18} column (Waters, Milford, MA) was held at 25 °C ± 1 °C with a column oven. A mobile phase gradient (water/acetonitrile/acetic acid) and a flow rate of 1.0 mL/min were used. Positive ion electrospray mass spectrometry was used for detection of the terpene lactones. Quantification was based on monitoring ions (*m/z*) at 327 (bilobalide), 409 (ginkgolide A), 425 (ginkgolides J and B), 441 (ginkgolide C) and 303 (hesperitin); hesperitin was used as the internal standard. For LC/MS Method 3, a 250 mm x 4.6 mm Xterra C_{18} column (Waters, Milford, MA) was held at 25 °C ± 1 °C with a column oven. A mobile phase gradient (water/acetonitrile/acetic acid) and a flow rate of 1.0 mL/min were used. Negative ion atmospheric pressure chemical ionization mass spectrometry was used for detection of the terpene lactones. Quantification was based on monitoring ions (*m/z*) at 325 (bilobalide), 467 (ginkgolide A), 423 (ginkgolides J and B), 439 (ginkgolide C), 483 (ginkgolide J), and 301 (hesperitin). Components are identified as follows: bilobalide (BB), ginkgolide-A (G-A), ginkgolide-B (G-B), ginkgolide-C (G-C), ginkgolide-J (G-J), limonin (L), hesperitin (H); hesperitin was used as the internal standard.

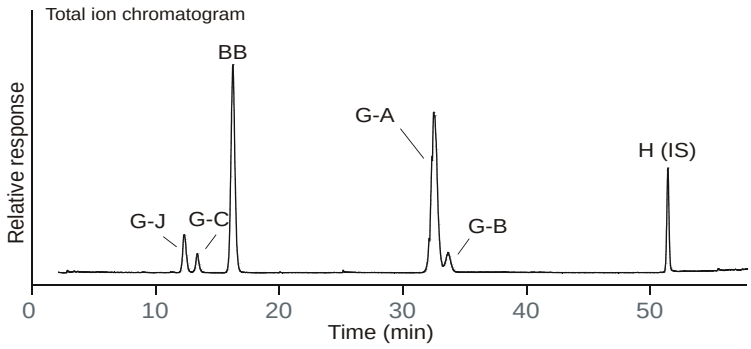
(a) LC/MS method 1



(b) LC/MS method 2



(c) LC/MS method 3



Appendix C1. Thin layer chromatography as provided by CAMAG; application note F16B for flavonoids. Stationary phase: HPTLC silica gel 60 F_{254} (Merck); mobile phase: ethyl acetate, acetic acid, formic acid, and water.

Prior to derivatization

Image under UV 254 nm

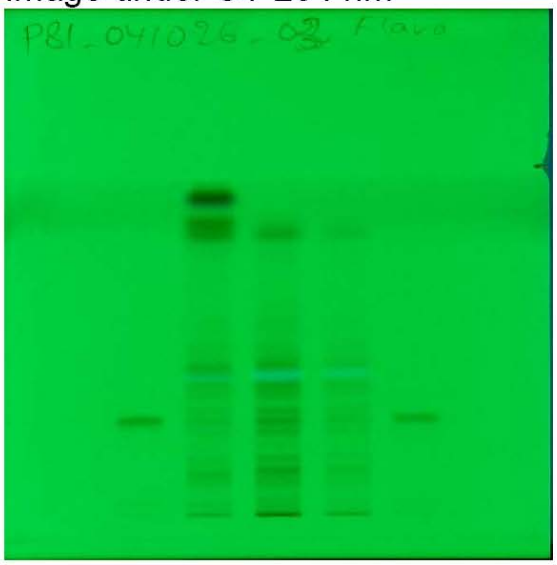
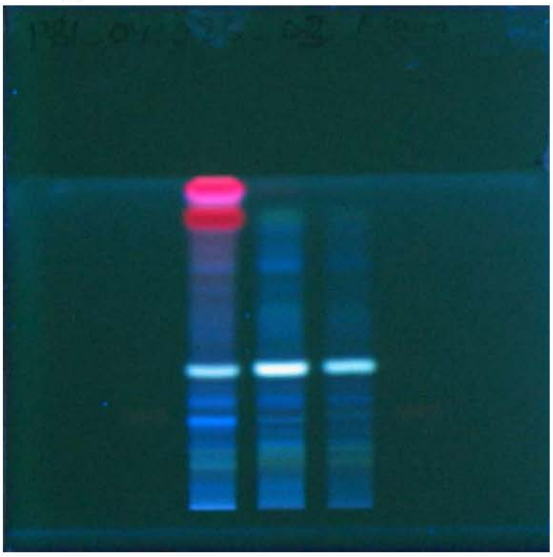


Image under UV 366 nm



After derivatization with Natural Products reagent + PEG

Image under UV 366 nm, NP

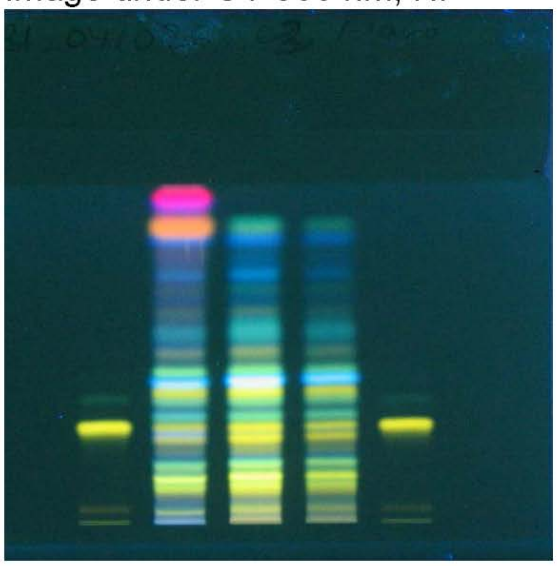
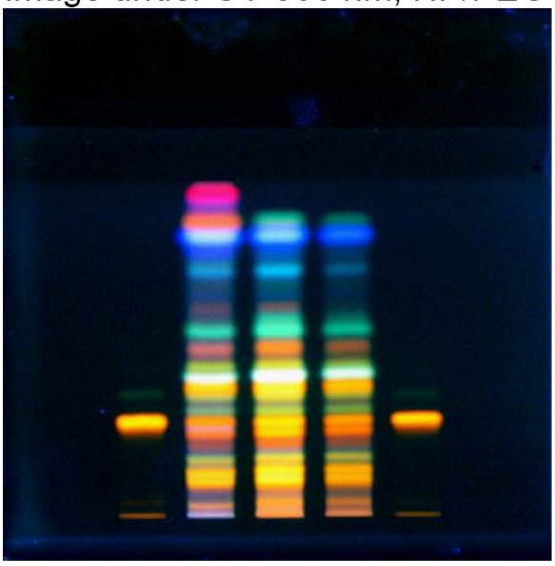


Image under UV 366 nm, NP/PEG



1: Rutin (1.5 mg/ 10 mL), 6 µL
2: *Ginkgo biloba* (Leaves) NIST SRM 3246 (1 g/ 10 mL), 5 µL
3: *Ginkgo biloba* (Extract) NIST SRM 3247 (100 mg/ 10 mL), 5 µL
4: Ginkgo-Containing Tablets NIST SRM 3248 (200 mg/ 10 mL), 5 µL
5: Rutin (1.5 mg/ 10 mL), 6 µL

Appendix C2. Thin layer chromatography as provided by CAMAG; application note F16A for ginkgolides. Stationary phase: HPTLC silica gel 60 F_{254} (Merck); mobile phase: ethyl acetate, acetic acid, formic acid, and water.

After derivatization with acetic anhydride

Image under UV 254 nm

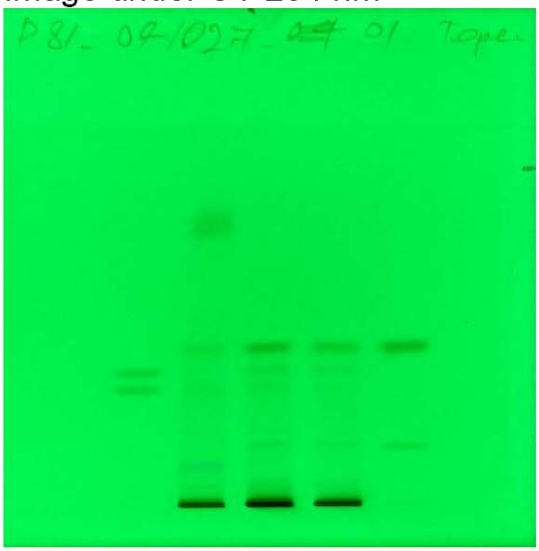
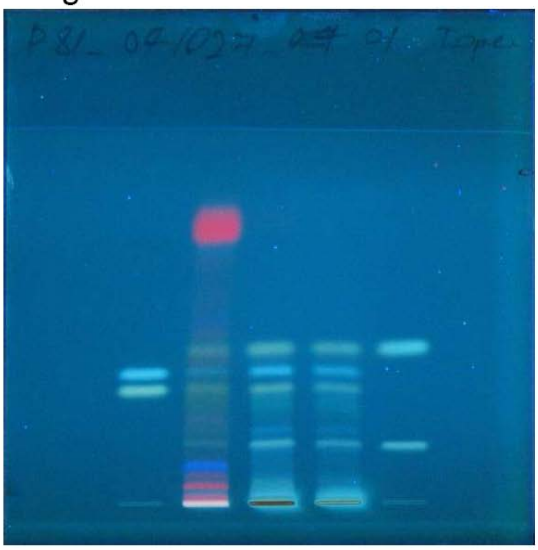


Image under UV 366 nm



1: Ginkolide A and B (1 mg/ mL), 3 µL each
2: *Ginkgo biloba* (Leaves) NIST SRM 3246 (1 g/ 10 mL), 5 µL
3: *Ginkgo biloba* (Extract) NIST SRM 3247 (100 mg/ 10 mL), 15 µL
4: Ginkgo-Containing Tablets NIST SRM 3248 (200 mg/ 10 mL), 25 µL
5: Ginkolide C and bilobalide (1 mg/ mL), 3 µL each

Appendix C3. Thin layer chromatography as provided by CAMAG; application note F16C for ginkgolic acid. Stationary phase: HPTLC silica gel 60 F_{254} (Merck); mobile phase: ethyl acetate, acetic acid, formic acid, and water.

No derivatization

Image under UV 254 nm

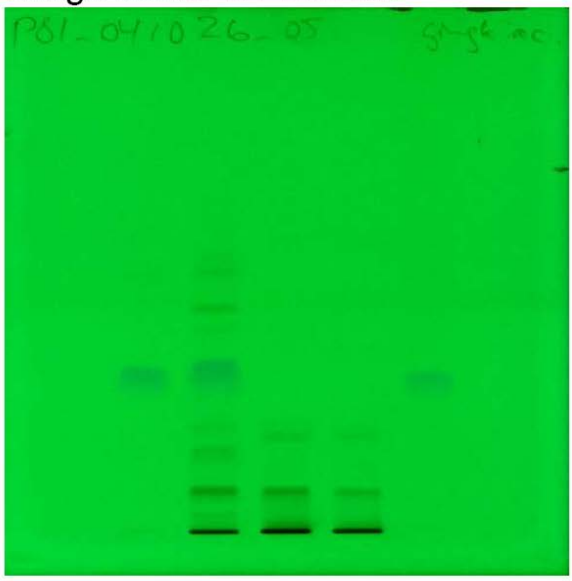
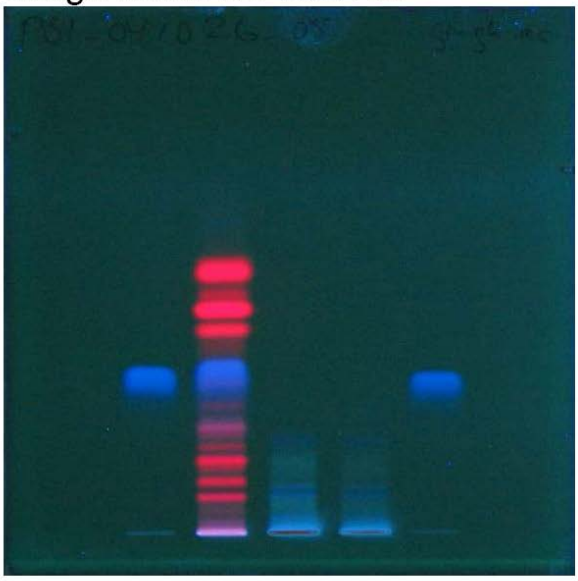


Image under UV 366 nm



1: Ginkgolic acid (1 mg/ 10 mL), 10 µL
2: *Ginkgo biloba* (Leaves) NIST SRM 3246 (1 g/ 10 mL), 4 µL
3: *Ginkgo biloba* (Extract) NIST SRM 3247 (100 mg/ 10 mL), 10 µL
4: Ginkgo-Containing Tablets NIST SRM 3248 (200 mg/ 10 mL), 15 µL
5: Ginkgolic acid (1 mg/ 10 mL), 10 µL