National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 3247

Ginkgo biloba (Extract)

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 biloba* extracts and similar matrices. This SRM can also be used for quality assurance when assigning values to in-house control materials. SRM 3247 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 3247 consists of five bottles, each containing approximately 1 g of extract.

The development of SRM 3247 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 Mass Fraction Values: NIST mass fraction values of flavonoid aglycones and terpene lactones, and lead in SRM 3247, reported on a dry-mass basis, are reported in Tables 1 and 2. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1]. Analyses for value assignment were performed by NIST 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 measurements made by collaborating laboratories; the associated uncertainties are expanded uncertainties at the 95 % level of confidence [2-4].

Reference Mass Fraction Values: Reference mass fraction values for arsenic and cadmium in SRM 3247, reported on a dry-mass basis, are provided in Table 3. A NIST reference value is a non-certified value that is the best estimate of the true value based on available data; however, the values do not meet the NIST criteria for certification [1] and are 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.

Expiration of Value Assignment: The certification of **SRM 3247** is valid, within the measurement uncertainty specified, until **30 October 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 Value Assignment: NIST will monitor this SRM over the period of its value assignment. If substantive technical changes occur that affect the value assignment 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.

Technical consultation from these agencies was provided by J. Betz (NIH ODS) and A. NguyenPho (FDA CDER).

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

Carlos A. Gonzalez, Chief
Chemical Sciences Division

Gaithersburg, MD 20899
Certificate Issue Date: 10 February 2021
Certificate Revision History on Page 6

Steven J. Choquette, Director
Office of Reference Materials

Analytical measurements at NIST were performed by, S.E. Long, K.E. Murphy, C.A. Rimmer, and L.J. Wood of the NIST Chemical Sciences Division and S.B. Howerton, B.J. Porter, K. Putzbach M.S. Rearick, D. Hancock, and R.L. Zeisler formerly of NIST. Analyses for value assignment were also performed by C. Scriver and L. Yang of the National Research Council Canada (NRCC; Ottawa, ON); Mariann 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 3247 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 in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

NOTICE AND WARNING TO USERS

SRM 3247 IS INTENDED 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: This material is a hard resin and the bottle may contain one or several solid pieces of ginkgo extract resin. Prior to analysis of a test portion, contents of the bottle should be ground and then mixed thoroughly. For certified values to be valid, test portions equal to or greater than 50 mg for flavonoids, 0.2 g to 1 g for terpene lactones, and 0.25 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.

Determination of Moisture: Moisture content of SRM 3247 was determined by (1) freeze-drying to constant mass over 14 d; (2) drying over magnesium perchlorate in a desiccator at room temperature for 19 d; and (3) drying for 24 h in a forced-air oven at 50 °C. Unweighted results obtained using all three techniques were averaged to determine a conversion factor of 0.9811 g 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 0.9720 as determined by drying two 1 g samples over magnesium perchlorate for 20 d. 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.

SOURCE, PREPARATION, AND ANALYSIS[(1)]

Material Acquisition and Preparation: Approximately 7 kg of *Ginkgo biloba* extract, prepared according to the German Pharmacopoeia (non-clinical) was received from the manufacturer. The 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 mass fractions of flavonoids in SRM 3247 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 was used for the value assignment of the mass fractions 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 3247.

[(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 a single sample preparation method (dissolution) and two LC methods with ultraviolet absorbance (UV) and mass spectrometric (MS) detection, respectively. 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.

Dissolution: Approximately 50 mg portions of SRM 3247 were combined with a measured mass of internal standard solution (hesperitin) and were dissolved in methanol with the aid of ultrasonic agitation.

Hydrolysis: After dissolution, 30 g of the solution 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 with UV Absorbance Detection: A C18 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 with Mass Spectrometric Detection: A C18 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 (quercetin, hesperetin), 317 (isorhamnetin), and 287 (kaempferol). Hesperitin was 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 3247 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 a single method for sample preparation (dissolution) and two different LC methods with mass spectrometric detection (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 a single sample preparation method (dissolution) and two LC/MS methods. Five (Method 1, below) or six (Method 2, below) independently prepared five-component calibration solutions were used for each of the methods. Calibrants 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 the calibrants (three injections of the five or six calibrant solutions, respectively).

Dissolution: Weighed aliquots of either 20 mg portions or 1 g portions of SRM 3247 were combined with a measured mass of internal standard solution (hesperitin or limonin, respectively) and were dissolved in methanol with the aid of ultrasonic agitation.

LC with Mass Spectrometric Detection (LC/MS) Method 1: A C12 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*) at 344 (bilobalide), 426 (ginkgolide A), 442 (ginkgolides J and B), 458 (ginkgolide C), and 488 (limonin). Limonin was used as the internal standard. A typical separation is provided in Appendix B.

LC with Mass Spectrometric Detection (LC/MS) Method 2: A C18 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*) at 327 (bilobalide), 409 (ginkgolide A), 425 (ginkgolides J and B), 441 (ginkgolide C), and 303 (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 3247 were the potentially toxic contaminants arsenic, cadmium, lead, and mercury. Value assignment of the mass fractions of toxic elements in SRM 3247 was based on the combination of measurements at NIST using a single analytical method and results from one 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 lead and hydride generation graphite furnace atomic absorption spectrometry (HG GFAAS) for the determination of arsenic. NRCC analyzed six subsamples of SRM 3247.

NIST Analyses for Elements: Arsenic was determined by using instrumental neutron activation analysis (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 x 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 0.25 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 the 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 3247 to estimate the uncertainty due to interference [6]. The sample was evaporated to dryness with concentrated hydrochloric acid to convert residual salts from the nitrate to the chloride form. The sample was redissolved in a mixture of hydrochloric and hydrofluoric acids, separated using anion chromatography, evaporated, and redissolved in nitric acid. There was a 4 % difference in determined Cd concentration between the separated and unseparated samples of SRM 3247.

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]. (A value was not assigned for mercury because of inhomogeneity, as described below).

Homogeneity Assessment: The homogeneity of flavonoids and terpene lactones in SRM 3247 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. Mercury appeared to be inhomogeneously distributed in 0.25 g samples across the samples tested, ranging from 0.29 ng/g to 4.5 ng/g; therefore, a value was not assigned. Other measurands were treated as though they were homogeneously distributed, although homogeneity was not assessed.

Value Assignment: 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.

Supplemental Information: In addition to the analyses described above, further characterization of SRM 3247 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 3247 and are not intended for use in identifying extracts of *Ginkgo biloba*.

Table 1. Certified Mass Fraction Values for Flavonoid Aglycones and Terpene Lactones in SRM 3247[a]

	Mass Fraction (mg/g)
Quercetin[b,c,d,e,f]	45.1 ± 4.6
Kaempferol[b,c,d,f]	40.8 ± 3.0
Isorhamnetin[b,c,d,e,f]	10.8 ± 1.3
Total Aglycones[b,c,d,f]	96.8 ± 8.3
Ginkgolide A[g,h]	11.6 ± 1.7
Ginkgolide B[g,h]	5.92 ± 0.45
Ginkgolide C[g,h]	12.4 ± 1.4
Ginkgolide J[g,h]	3.9 ± 1.5
Bilobalide[g,h]	28.5 ± 2.1
Total Terpene Lactones[g,h]	62.4 ± 5.7

[a] Each certified mass fraction value is an equally weighted mean of results from analytical methods carried out at NIST and at collaborating laboratories. 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 is believed to lie within the interval $x \pm U_{95\%}(x)$ with 95 % confidence. To propagate this uncertainty, treat the certified value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [2-4]. The measurand is the total mass fraction for each analyte listed in Table 1 on a dry-mass basis. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per gram).
[b] NIST LC/UV
[c] NIST LC/MS
[d] ChromaDex LC/UV
[e] NSF International LC/UV
[f] AOAC Collaborative Study
[g] Two NIST LC/MS Methods
[h] Eurofins LC/ELSD

Table 2. Certified Mass Fraction Value for Lead in SRM 3247[a]

	Mass Fraction (mg/kg)
Lead (Pb)[b,c]	4.273 ± 0.031

[a] The certified mass fraction value for lead is an equally weighted mean of the results from NIST and NRCC. The value is 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 is believed to lie within the interval $x \pm U_{95\%}(x)$ with 95% confidence. To propagate this uncertainty, treat the certified value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [2-4]. The measurand is the total mass fraction for lead listed in Table 2 on a dry-mass basis. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per kilogram).
[b] NIST ID ICP-MS
[c] NRCC ID ICP-MS

Table 3. Reference Mass Fraction Values for Arsenic and Cadmium in SRM 3247[a]

	Mass Fraction (mg/kg)
Arsenic (As)[b,c]	0.314 ± 0.012
Cadmium (Cd)[d]	0.00753 ± 0.00077

[a] Each reference mass fraction value, reported on a dry-mass basis, is an equally weighted mean of the results from NIST and NRCC (where available). Values are expressed as $x \pm U_{95\%}(x)$, where x is the reference value and $U_{95\%}(x)$ is the expanded uncertainty of the reference value. The method-specific value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with 95 % confidence. To propagate this uncertainty, the reference value should be treated as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [2-3]. The measurands are the total mass fraction of each analyte in Table 3. The reference mass fraction values are metrologically traceable to the to the SI unit of mass, expressed as micrograms per kilogram.
[b] NIST INAA
[c] NRCC HG-GFAAS
[d] NIST ID ICP-MS

REFERENCES

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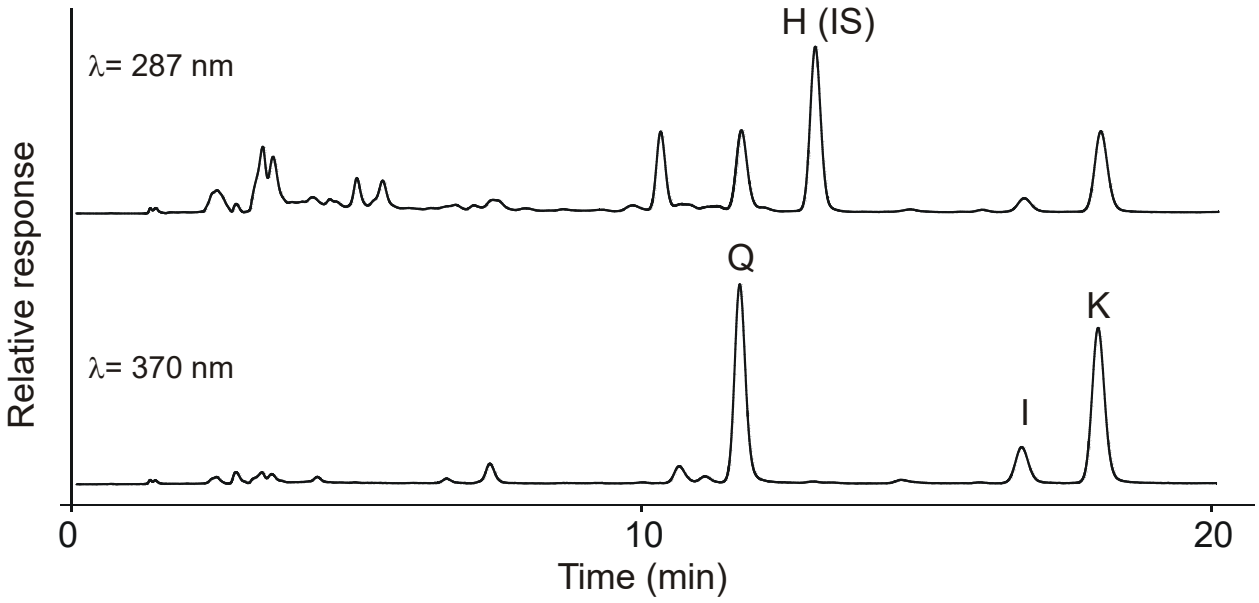
[7] Christopher, S.J.; Long, S.E.; Rearick, M.S.; Fassett, J.D.; *Development of Isotope Dilution Cold Vapor Inductively Coupled Plasma Mass Spectrometry and Its Application to the Certification of Mercury in NIST Standard Reference Materials*; Anal. Chem., Vol. 73, pp. 2190–2199 (2001).

Certificate Revision History: 10 February 2021 (Update of "Use" section with description of material and preparation prior to analysis; editorial changes); 22 May 2019 (Change of expiration date; editorial changes); 11 August 2014 (Extension of certification period; editorial changes); 27 July 2007 (Original certificate date).

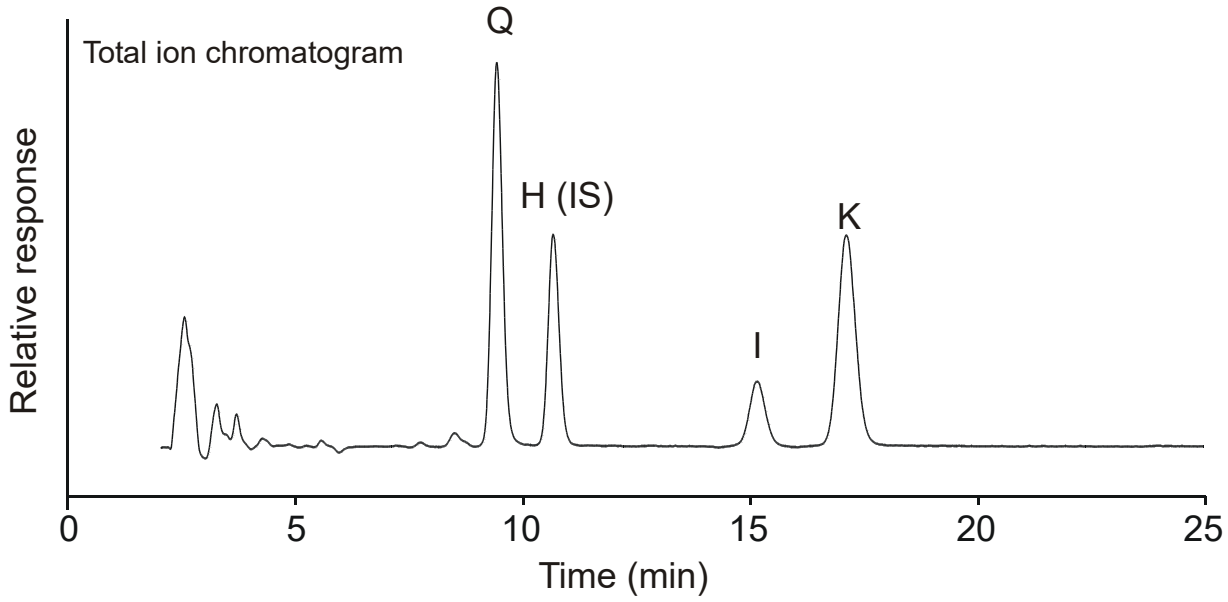
Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; e-mail srminfo@nist.gov; or via the Internet at https://www.nist.gov/srm.

Typical chromatograms from the analysis of flavonoid aglycones in SRM 3247 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 (0.5 µm). A new precolumn and filter was 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 (C18 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

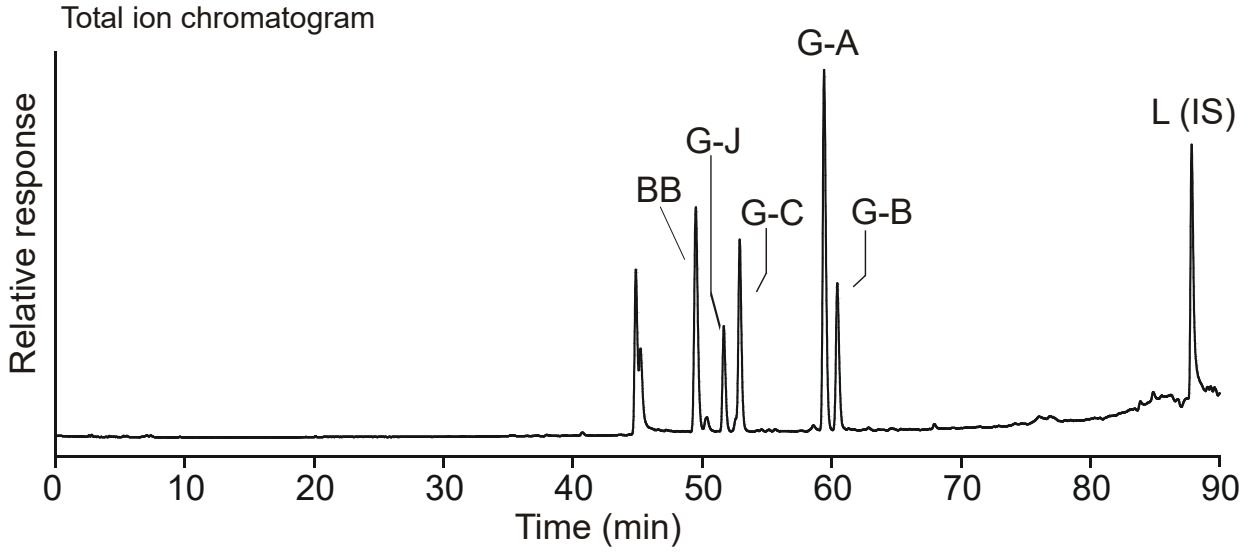


(b) LC/MS method 2

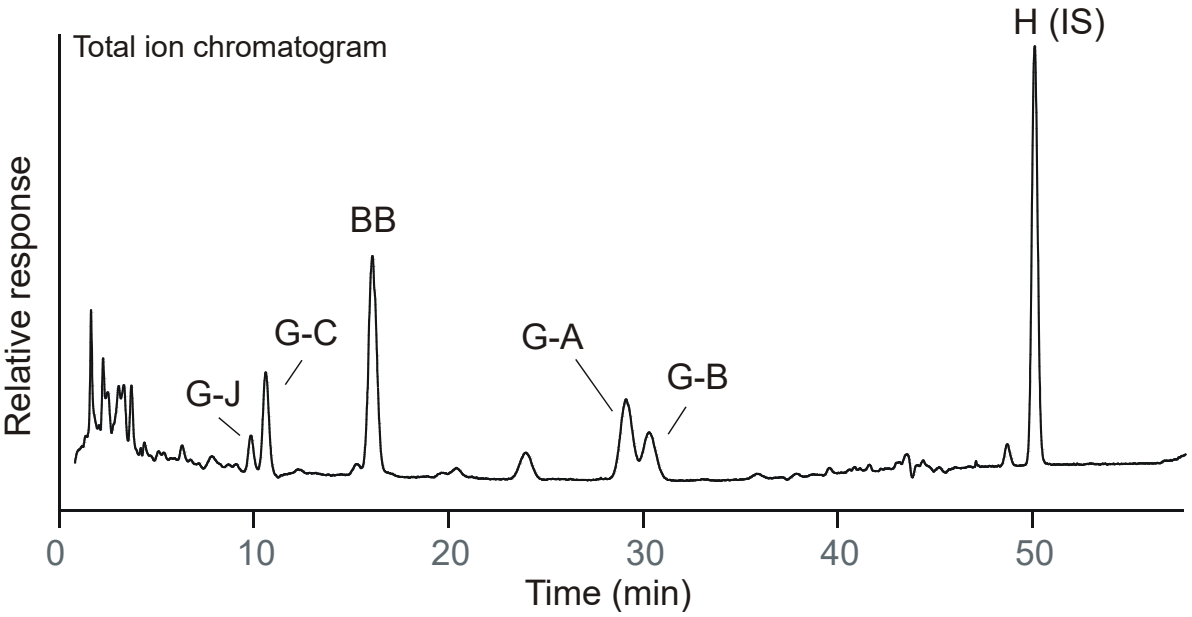


Typical chromatograms from the analysis of ginkgolides and bilobalide in SRM 3247 by using: (a) LC/MS method 1 and (b) LC/MS method 2. For LC/MS Method 1, a 250 mm x 4.6 mm Synergi-Max RP column (Phenomenex, Madrid, CA) and Synergi-Max RP guard column (Phenomenex) were held at 25 °C ± 1 °C with a column 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 C18 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). 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). Limonin and hesperitin were added as internal standards.

(a) LC/MS method 1



(b) LC/MS method 2



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

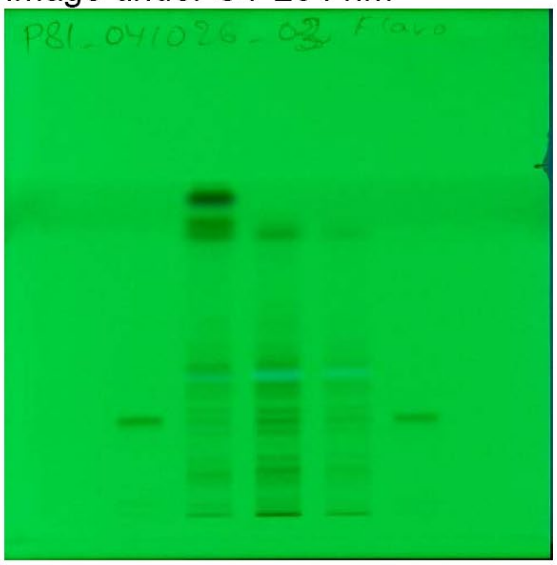
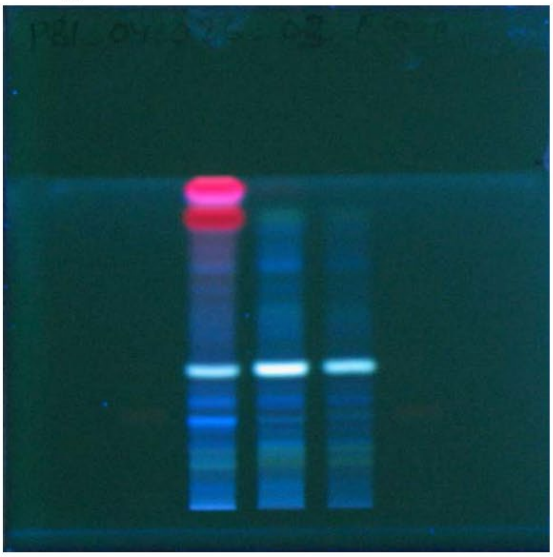


Image under UV 366 nm



After derivatization with Natural Products reagent + PEG

Image under UV 366 nm, NP

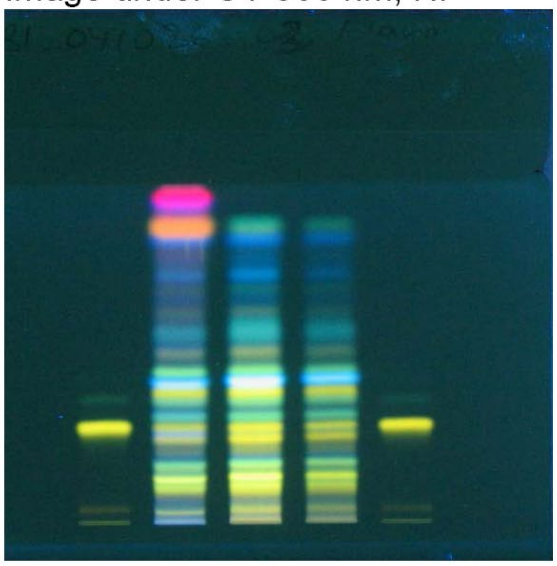
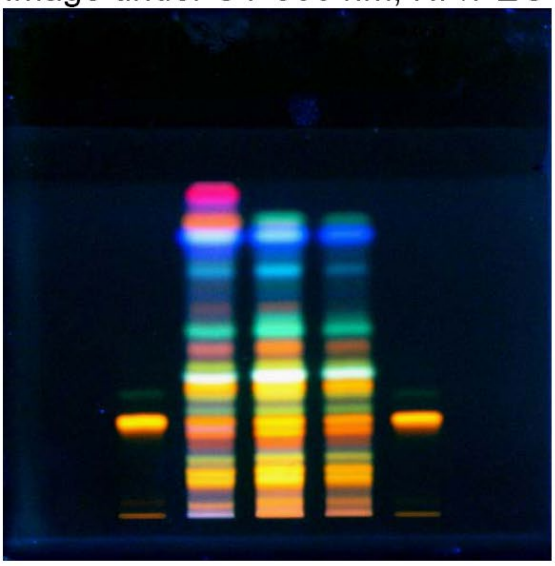


Image under UV 366 nm, NP/PEG



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

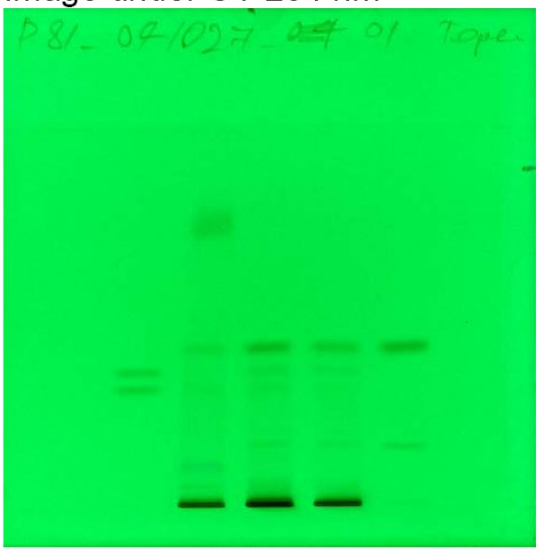
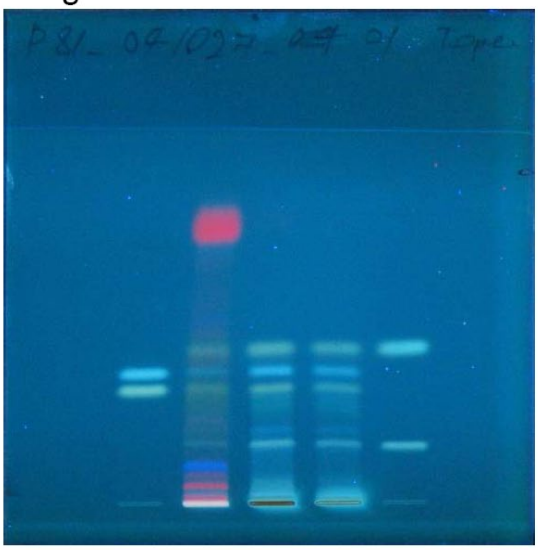


Image under UV 366 nm



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

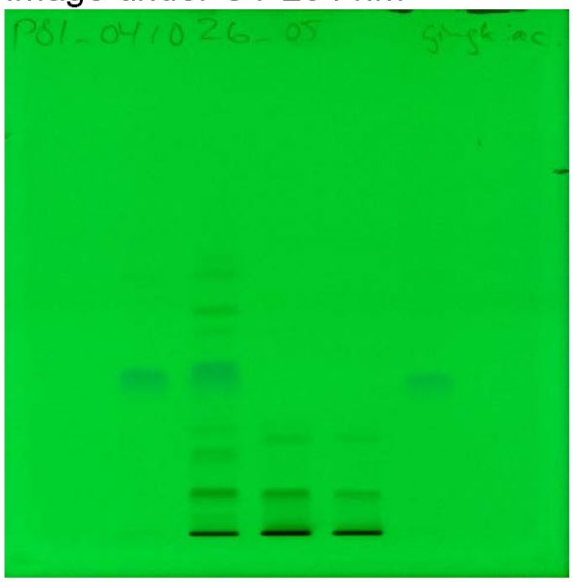
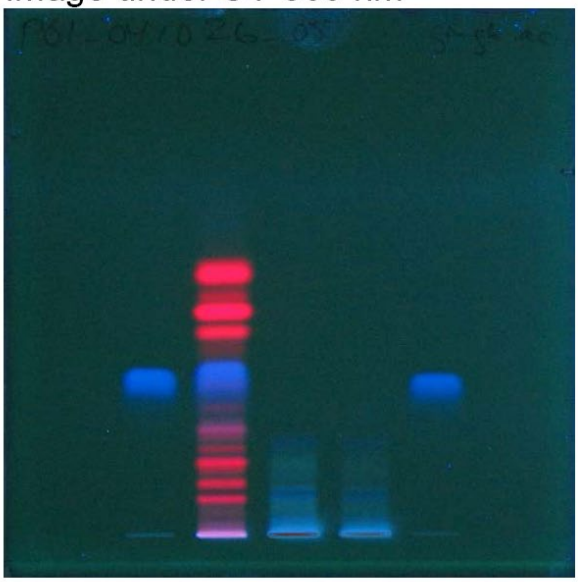


Image under UV 366 nm



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