National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 3240

Ephedra sinica Stapf Aerial Parts

Standard Reference Material (SRM) 3240 is intended primarily for use in validating analytical methods for the determination of ephedrine alkaloids and toxic elements in *Ephedra sinica* and similar matrices. This SRM can also be used for quality assurance when assigning values to in-house control materials. SRM 3240 is part of a suite of ephedra dietary supplement SRMs that have been developed to cover a range of natural matrices and ephedrine alkaloid levels. A unit of SRM 3240 consists of ten bottles of ground aerial parts, each containing approximately 5 g of material.

Certified Concentration 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 accounted for [1]. The certified concentration values of selected ephedrine alkaloids and elements 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 individual 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 Concentration 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 ephedrine alkaloids and elements are provided in Tables 3 and 4.

Expiration of Value Assignment: The value assignment of this SRM is valid until **31 March 2014**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate. Value assignment is nullified if the SRM is damaged, contaminated, or 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) will facilitate notification.

The development of SRM 3240 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 Food Safety and Applied Nutrition (CFSAN) and Center for Drug Evaluation and Research (CDER).

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 Analytical Chemistry Division.

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

Analytical measurements at NIST were performed by T.A. Butler, T. Ihara, S.E. Long, E.A. Mackey, K.E. Murphy, K.W. Phinney, B.J. Porter, L.C. Sander, M.B. Satterfield, R.D. Vocke, Jr., and L.J. Wood of the NIST Analytical Chemistry Division. Analyses for value assignment were also performed by C. Fraser, G. Gardner, J.W. Lam, M. McCooeye, C. Scriver, and L. Yang of the National Research Council Canada (Ottawa, ON); D.L. Anderson, J. Cheng, M.L. Gay, and W. Mindak at the FDA's Center for Food Safety and Applied Nutrition (College Park, MD); and S. Mitvalsky and M. Roman at ChromaDex, Inc. (Clearwater, FL).

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

Support for the development of SRM 3240 was provided in part by the NIH Office of Dietary Supplements and the FDA Center for Food Safety and Applied Nutrition (CFSAN) and the Center for Drug Evaluation and Research (CDER). Technical consultation from these agencies was provided by J. Betz (NIH-ODS), A. NguyenPho (FDA CDER), and G. Ziobro (FDA CFSAN).

The support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

NOTICE AND WARNING TO USERS

Note: This is a natural ephedra plant material and is therefore exempt from requirements of Title 21 Code of Federal Regulations Section 1310 (21 CFR 1310).

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

WARNING: FOR LABORATORY USE ONLY. NOT FOR HUMAN CONSUMPTION.

INSTRUCTIONS FOR USE

Prior to removal of a test portion for analysis, the contents of a bottle of material should be mixed thoroughly. Test portions used for NIST analyses described below were 0.5 g and 1 g for ephedrine alkaloids, 0.2 g for arsenic and mercury, and 1 g for cadmium.

PREPARATION AND ANALYSIS[1]

Material Acquisition and Preparation

A single year's harvest of *Ephedra sinica* Stapf from a single field in China was acquired in 2002 from Jinke Group USA, Inc. (Diamond Bar, CA) through Modern Nutrition and Biotech (Appleton, WI). The crop was examined by a Chinese taxonomist (Xian-Chun Zhang, Institute of Botany, Chinese Academy of Sciences, Beijing, China) who verified its identity, and representative herbarium specimens were collected at time of flower and shipped with the dried botanical following harvest in the same year. The herbarium sheets (Appendix A) were deposited at the herbarium of the FDA CFSAN (FDA Accession No. 1221) and the Missouri Botanical Garden (Herbarium Sheet No. 5827116; http://www.mobot.org/MOBOT/research/diversity/herbarium/compendium_model.aspx?id=3; click on *Ephedra sinica* Specimen 1). While still in China, the plant material (aerial parts) was dried, powdered, sieved to 177 μm (80 mesh), and sterilized using a 6 kGy dose of ^{60}Co. Approximately 100 kg of the dried powdered plant material was shipped to NIST; the botanical raw material was subsequently transferred to ChromaDex, Inc. (Santa Ana, CA) where it was blended and bottled under nitrogen in amber high-density polyethylene bottles with polypropylene screw caps. After bottling, the materials were irradiated by ^{60}Co to an absorbed dose of 12.8 kGy to 15.4 kGy.

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[1]Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Analytical Approach for Determination of Ephedrine Alkaloids

Value assignment of the concentrations of the ephedrine alkaloids in SRM 3240 was based on the combination of measurements from different analytical methods at NIST and at three collaborating laboratories. A total of nine sets of measurements were used for the value assignment of the concentrations of ephedrine alkaloids. 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), mass spectrometry (MS), tandem mass spectrometry (MS/MS), and a capillary electrophoresis (CE) method as described below. Results for ephedrine alkaloids were provided by three collaborating laboratories: National Research Council Canada (NRCC), FDA, and ChromaDex. NRCC provided results from three analytical methods: LC/UV, LC/MS/MS, and high-field asymmetric waveform ion mobility spectrometry (FAIMS). FAIMS is a mass spectrometry technique that provided results without using a chromatographic separation [5]. FDA results were based on LC/MS/MS [6], and ChromaDex results were based on LC/UV [7]. Two collaborating laboratories analyzed a minimum of six subsamples (one from each of six bottles or two from each of three bottles); one laboratory analyzed one subsample from three bottles of SRM 3240. The analytical methods for NIST and the collaborating laboratories are described in detail in reference 8.

NIST Analyses for Ephedrine Alkaloids

Ephedrine alkaloids were measured by using combinations of two sample preparation methods, three LC methods, and one CE method as described below and in detail in reference 8. Four independently prepared calibrants were used for each of the methods. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the alkaloids 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 (typically duplicate analysis of four calibrant solutions, n = 8).

Sonication Extraction: Six 1 g portions of the SRM were placed in 50 mL polyethylene centrifuge tubes or glass pressurized-fluid extraction tubes, followed by the addition of a measured mass of internal standard solution. Approximately 30 g 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 90 min. At the completion of the sonication extraction, the samples were centrifuged or allowed to settle, and an aliquot of the supernatant solution was passed through a 0.45 μm × 2.5 cm syringe filter. Samples prepared by this approach were analyzed by LC/UV or LC/MS/MS.

Soxhlet Extraction: Ten 1 g portions of the SRM were weighed into glass-fritted Soxhlet thimbles each containing an approximate 1 cm layer of diatomaceous earth (Hydromatrix, Isco Corp., Lincoln, NE). After stirring the sample, additional diatomaceous earth was added (approximately 1 cm). A measured mass of internal standard solution (ephedrine-d_3) was transferred to the Soxhlet thimble. The samples were extracted with approximately 200 mL methanol for at least 18 h. Extracts were concentrated, passed through a 0.45 μm × 2.5 cm syringe filter, and analyzed. Samples prepared by this approach were analyzed by LC/MS.

LC with UV Absorbance Detection (LC/UV): An isocratic LC method with a methanol/phosphate buffer mobile phase was used for LC/UV determination of the alkaloids, similar to the method of Roman [7]. A 250 mm × 4.6 mm alkylphenyl bonded-phase column (Synergy Polar RP, Phenomenex, Torrance, CA) was used with a precolumn and an in-line filter. Column temperature was controlled at 29.0 ºC ± 0.5 ºC with a circulating-fluid column jacket and water bath. The mobile phase flow rate was set at 1.5 mL/min, and detection was at 208 nm. Terbutaline was used as the internal standard for LC/UV measurements. A typical separation is provided in Appendix B.

LC with Mass Spectrometric Detection (LC/MS): A 250 mm × 4.6 mm phenyl bonded-phase column (YMC Phenyl, Waters, Inc., Milford, MA) was used at ambient temperature (21 °C ± 1 °C) with an isocratic mobile phase (water/methanol/acetic acid/ammonium acetate) at 1.0 mL/min. The mass spectrometer was operated in positive ion, atmospheric pressure ionization, electrospray mode (API-ES). Quantification of the six alkaloids was based on monitoring ions (m/z) at 134 (norephedrine and norpseudoephedrine), 148 (ephedrine and pseudoephedrine), 180 (methylephedrine and methylpseudoephedrine), and 169 (ephedrine-d_3). Ephedrine-d_3 was used as the internal standard for LC/MS measurements. A typical separation is provided in Appendix B.

LC with Tandem Mass Spectrometric Detection (LC/MS/MS): Chromatographic conditions were similar to those used in the LC/MS method; however, the flow rate was reduced to 0.5 mL/min and column temperature was set at 30 °C ± 2 °C. A program was designed to measure each individual analyte using multiple reaction monitoring (MRM). The protonated precursor of each analyte was selected in the first quadrupole, these ions collisionally dissociated in the collision cell (the second quadrupole), and the predetermined fragment ions monitored in the third quadrupole. The following precursor and fragment ions (*m/z*) were monitored: 151.8 and 134.0 (norephedrine and norpseudoephedrine), 165.9 and 148.0 (ephedrine and pseudoephedrine), 179.9 and 162.0 (methylephedrine and methylpseudoephedrine), and 168.9 and 151.0 (ephedrine-d_3). Ephedrine-d_3 was used as the internal standard for LC/MS/MS measurements. A typical separation is provided in Appendix B.

Capillary Electrophoresis (CE): Portions (0.5 g) of the SRM were weighed into polyethylene centrifuge tubes, followed by the addition of a measured mass of internal standard solution (β-phenylethylamine hydrochloride) and approximately 18 mL of methanol. The samples were placed in an ultrasonic bath for 30 min, and the supernatant solution was passed through 0.2 μm nylon syringe filters. Electrophoretic measurements were performed on a CE system with a photodiode array detector (data collected at 210 nm) with a high-sensitivity UV detection cell. Three chiral CE methods (utilizing different cyclodextrin-based chiral selectors) were used to analyze the samples. The methods were sufficiently independent to provide slightly different selectivity, thereby reducing the likelihood of undetected peak overlap and providing additional confidence in the enantiomeric identity of the analytes. Separations were performed in unmodified fused silica capillaries maintained at 25 °C, and injections were performed by pressure. Applied voltages were in the range of 15 kV to 30 kV. A detailed discussion of the CE method is provided in reference 9. A typical separation is provided in Appendix B; note that only the (−)-ephedrine and (+)-pseudoephedrine enantiomers that are naturally occurring in *E sinica* were found in this material, indicating that the plant material was not altered through the addition of synthetic alkaloids.

Analytical Approach for Determination of Elements

The elements of primary interest for SRM 3240 were the potentially toxic contaminants arsenic, cadmium, lead, and mercury. Value assignment of the concentrations of toxic elements in SRM 3240 was based on the combination of measurements at NIST using a single analytical method and results from one or two collaborating laboratories (NRCC and FDA). 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 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. FDA provided results for cadmium, lead, and mercury using ICP-MS. FDA also provided results using prompt gamma activation analysis (PGAA) for additional elements including boron, calcium, carbon, chlorine, gadolinium, hydrogen, iron, magnesium, nitrogen, potassium, samarium, silicon, and sulfur. All collaborating laboratories analyzed a minimum of six subsamples (one from each of six bottles or two from each of three bottles) of SRM 3240.

NIST Analyses for Elements

Arsenic was measured by using instrumental neutron activation analysis (INAA). Individual disks were formed from 200 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} s^{-1} for a total of 2 h. Decay times were approximately 5 d to 7 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 line from [76]As.

For cadmium determinations, a single 1 g portion was taken from each of six bottles of the SRM. Isotopically enriched [111]Cd was added to the samples prior to digestion in quartz vessels with nitric acid using a high-pressure microwave system. The microwave digests were transferred to Teflon PFA (perfluoroalkoxy polymer) beakers, concentrated, treated with HF at 150 °C for 4 h, and the residue redissolved in 2 % nitric acid, and measurements were made by using ID ICP-MS [10].

For mercury determinations, a single 0.2 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 to contain an approximate ^{201}Hg concentration of 0.05 ng/g. Samples were allowed to degas overnight at 4 °C. Measurements were made by using cold-vapor mercury generation (using tin (II) chloride reductant) coupled with ID ICP-MS [11].

NIST Determination of Moisture

Moisture content of SRM 3240 was determined by (1) freeze-drying to constant mass over 7 days; (2) drying over magnesium perchlorate in a desiccator at room temperature for 5 days; and (3) drying in a forced-air oven at 85 °C for 4 h. Unweighted results obtained using all three techniques were averaged to determine a conversion factor of 0.9548 gram dry mass per gram as-received mass, which was used to convert NIST data from an as-received to a dry-mass basis. Collaborating laboratories converted their data to a dry-mass basis using their own moisture determinations. 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 ephedrine alkaloids was assessed at NIST by using the LC/UV method described above. There was no trend in ephedrine data across bottles for 1 g test portions of SRM 3240, therefore the alkaloid data were treated as though the alkaloids were homogeneously distributed in the material. The 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. In cases where NIST made measurements, the NIST means were averaged with the individual data set means provided by collaborating laboratories to obtain the assigned value. In cases where NIST did not make measurements, the mean of the data set means became the assigned value.

Supplemental Information

In addition to the analyses of the material described above, further characterization of SRM 3240 was provided using LC with absorbance detection and microscopy. The experimental procedures and the results are provided in Appendices C and D, respectively. These results are provided only as supplemental information to assist in identifying and characterizing SRM 3240.

Table 1. Certified Concentration Values for Ephedrine Alkaloids in SRM 3240[a]

Analyte	Mass Fraction (mg/g)		
Ephedrine[b,c,d,e,f,g,h,i,j]	11.31	±	0.76
Methylephedrine[b,c,d,f,g,h,i,j]	1.18	±	0.14
Pseudoephedrine[b,c,d,e,f,g,h,i,j]	3.53	±	0.26
Total Alkaloids[b,c,d,f,g,h,i,j]	17.0	±	1.2

[a] Each certified concentration value, expressed as a mass fraction on a dry-mass basis, is an equally weighted mean of results from eight or nine 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 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.

[b] NIST LC/UV

[c] NIST LC/MS

[d] NIST LC/MS/MS

[e] NIST CE

[f] FDA LC/MS/MS

[g] ChromaDex LC/UV

[h] NRCC FAIMS

[i] NRCC LC/UV

[j] NRCC LC/MS/MS

Table 2. Certified Concentration Values for Selected Elements in SRM 3240[a,b]

Element	Mass Fraction (mg/kg)		
Arsenic[c,d]	0.265	±	0.016
Cadmium[e,f,g]	0.0906	±	0.0039
Mercury[g,h]	0.0167	±	0.0005

[a] Each certified concentration value, expressed as a mass fraction on a dry-mass basis, is an equally weighted mean of the results from analyses by NIST and collaborating laboratories. The uncertainty in the certified value, calculated according to the method described in the ISO 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.

[b] A concentration value is not assigned for lead in SRM 3240. Analyses by collaborating laboratories showed that lead was not homogeneously distributed in this material, with concentrations ranging from 1.3 mg/kg to 16 mg/kg.

[c] NIST INAA

[d] NRCC HG-GFAAS

[e] NIST ID ICP-MS

[f] NRCC ID ICP-MS

[g] FDA ICP-MS

[h] NIST CV ID ICP-MS

Table 3. Reference Concentration Values for Ephedrine Alkaloids in SRM 3240[a]

Analyte	Mass Fraction (mg/g)
Methylpseudoephedrine[c,d,h,i]	0.046 ± 0.015
Norephedrine[b,c,d,e,f,g,h,i]	0.44 ± 0.09
Norpseudoephedrine[b,c,d,e,f,g,h,i]	0.65 ± 0.14

[a] Each reference concentration value, expressed as a mass fraction on a dry-mass basis, is an equally weighted mean of the results from four or eight 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 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.
[b] NIST LC/UV
[c] NIST LC/MS
[d] NIST LC/MS/MS
[e] FDA LC/MS/MS
[f] ChromaDex LC/UV
[g] NRCC FAIMS
[h] NRCC LC/UV
[i] NRCC LC/MS/MS

Table 4. Reference Concentration Values for Selected Elements in SRM 3240[a]

Element	Mass Fraction (%)
Calcium	2.69 ± 0.08
Carbon	45.0 ± 1.1
Hydrogen	5.51 ± 0.13
Nitrogen	1.58 ± 0.08

Element	Mass Fraction (mg/kg)
Boron	13.0 ± 0.4
Chlorine	4600 ± 120
Gadolinium	0.085 ± 0.016
Iron	457 ± 67
Magnesium	3380 ± 410
Potassium	5470 ± 120
Samarium	0.097 ± 0.015
Silicon	3600 ± 230
Sulfur	1770 ± 50

[a] Each reference concentration value, expressed as a mass fraction on a dry-mass basis, is the equally weighted mean of values provided by one collaborating laboratory using PGAA. The uncertainty in the reference value, calculated according to the method described in the ISO 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 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.

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Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-6776; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.
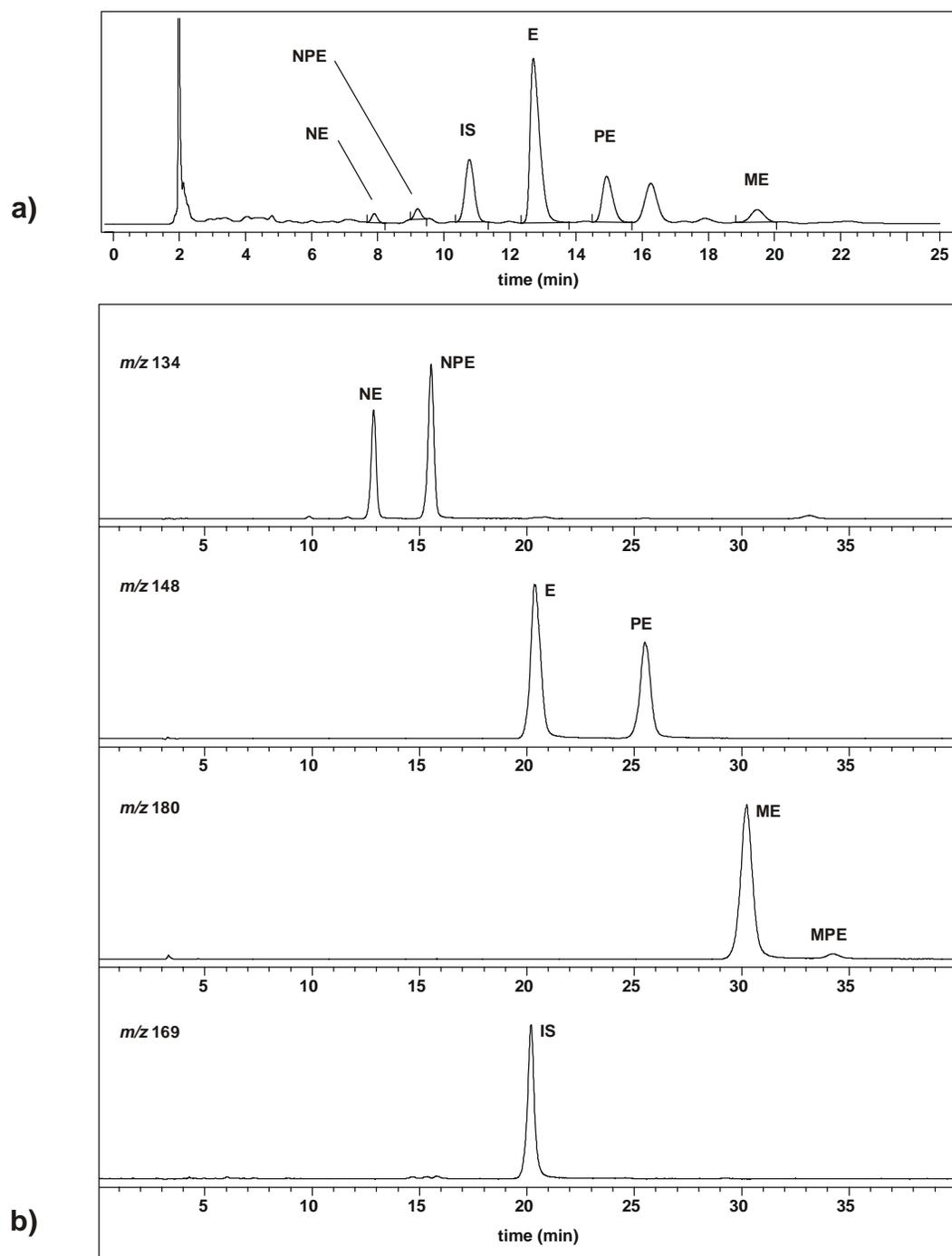
Photograph of a portion of the voucher specimen deposited at the Missouri Botanical Garden (Herbarium Sheet No. 5827116; http://www.mobot.org/MOBOT/research/diversity/herbarium/compendium_model.aspx?id=3; click on *Ephedra sinica* Specimen 1).
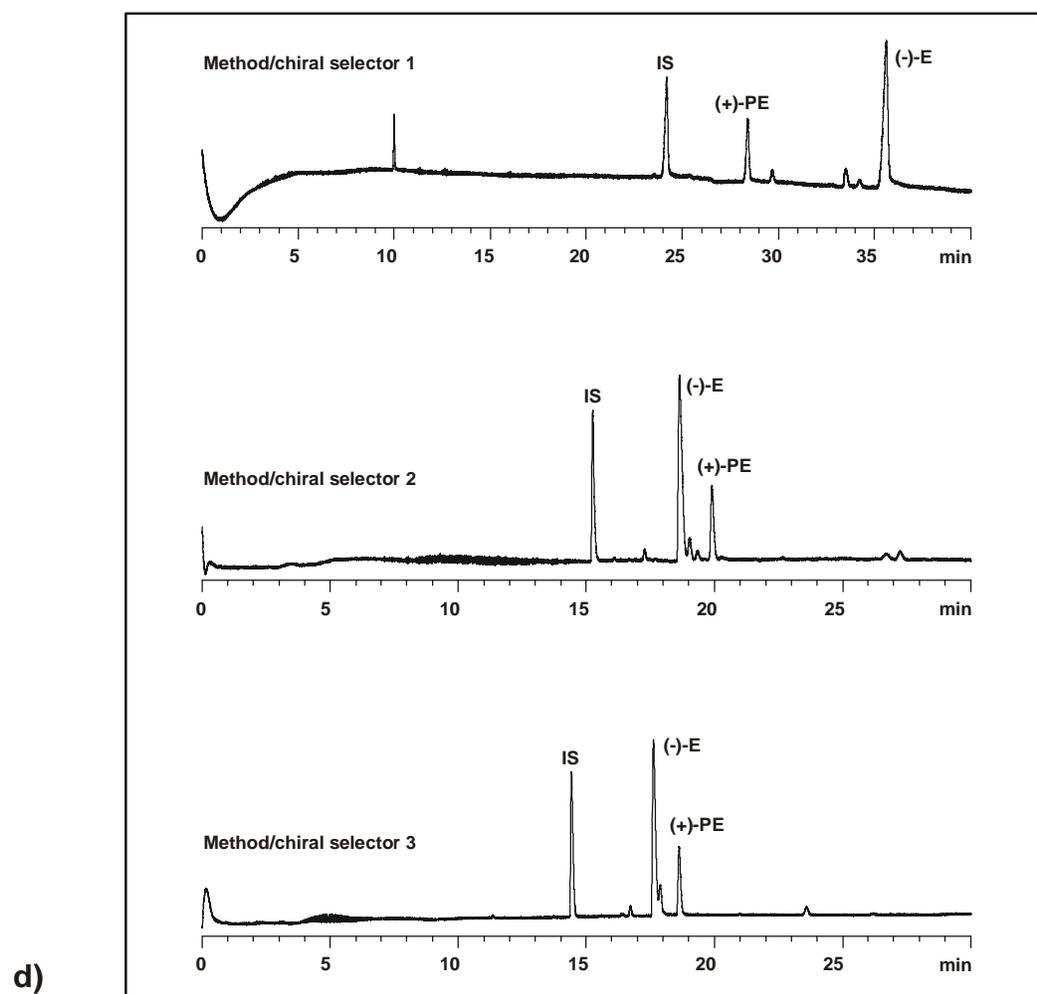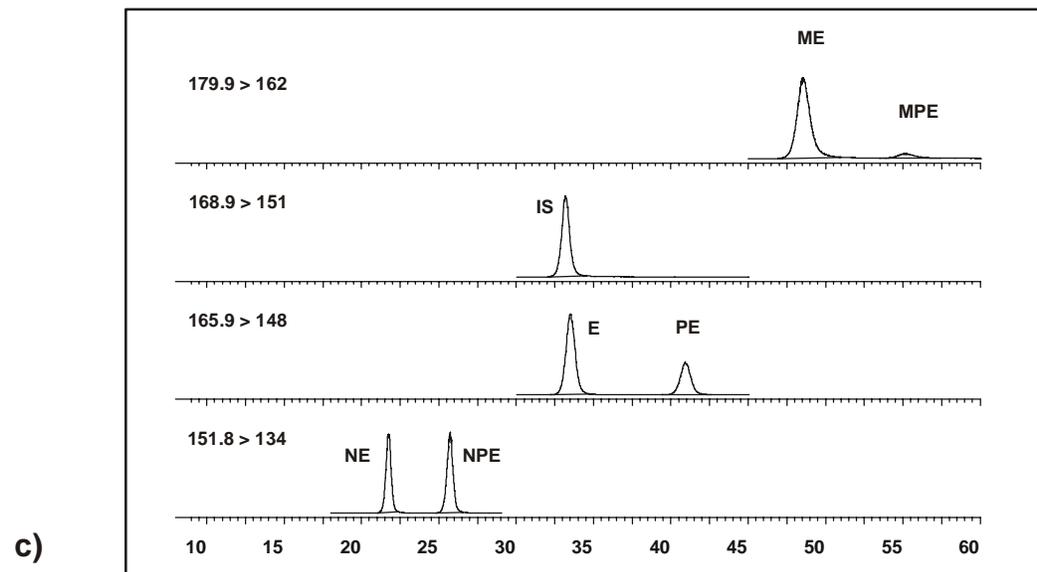
APPENDIX B

Typical chromatograms from the analysis of SRM 3240 by using a) LC/UV; b) LC/MS; c) LC/MS/MS; and d) CE. Components are identified as follows: norephedrine (NE); norpseudoephedrine (NPE); ephedrine (E); pseudoephedrine (PE); methylephedrine (ME); methylpseudoephedrine (MPE); internal standard (IS); (−)-ephedrine [(−)-E]; and (+)-pseudoephedrine [(+)-PE].
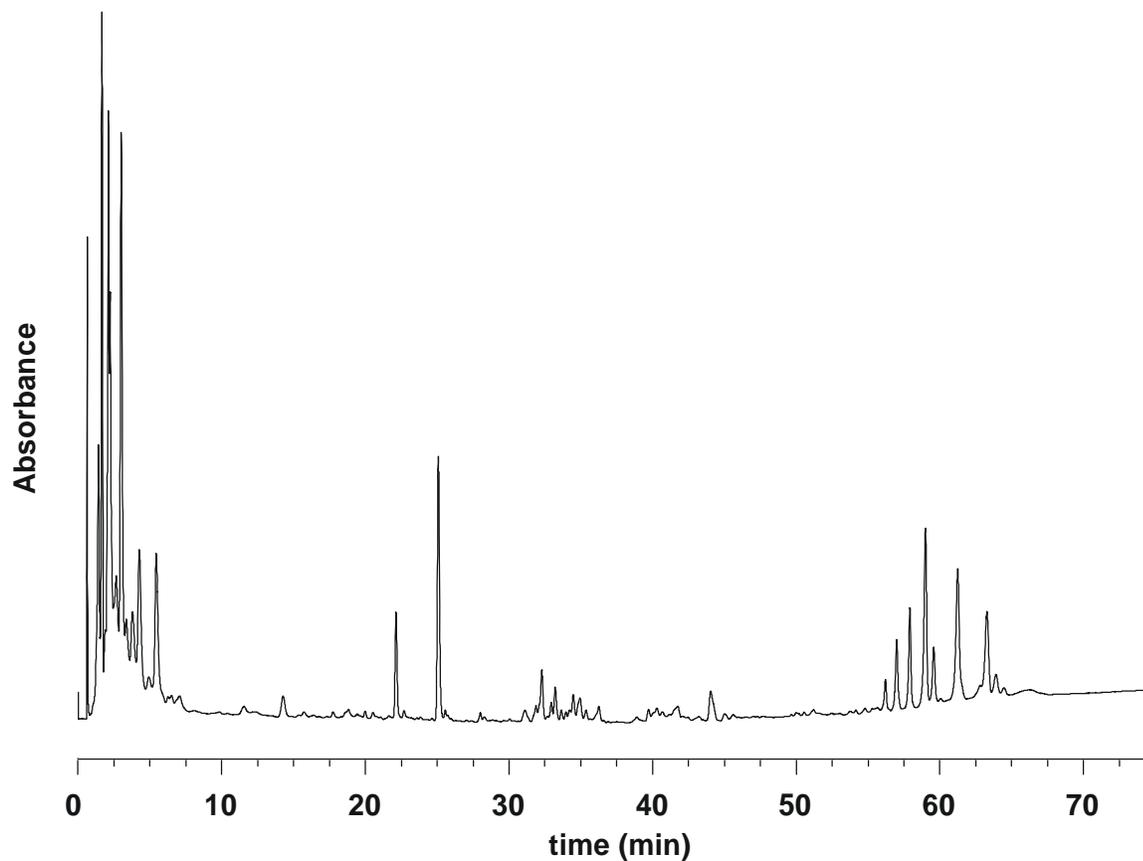
Typical chromatograms from the analysis of SRM 3240 by using a) LC/UV; b) LC/MS; c) LC/MS/MS; d) CE.

c)

d)

LC Fingerprint: A "fingerprint" chromatogram from the analysis of SRM 3240 *Ephedra sinica* Stapf Aerial Parts was created using the method published by Schaneberg et al. [12]. A 0.58 g sample of the SRM was extracted with 6 mL acetone by sonication for 15 min. The slurry was centrifuged for 5 min and decanted. This procedure was repeated twice and the three extracts were combined and evaporated to dryness. The extract was reconstituted in ethanol, and analyzed by gradient elution LC using a 4.6 mm × 150 mm, 5 μm particle size, C_{18} column (XTerra RP_{18}, Waters, Inc., Milford, MA) operated at 40 °C, with UV absorbance detection at 320 nm [7]. The mobile phase conditions consisted of an initial 10 min isocratic separation at 75 + 25 (volume fraction) water + acetonitrile, followed by a linear gradient to 100 % acetonitrile over 45 min. The end conditions were held for 20 min, for a total run time of 75 min. The flow rate was 1 mL/min, and the injection volume was 10 μL. This chromatogram is provided as additional information to assist in identifying and characterizing SRM 3240.

Microscopic study of SRM 3240 and cross section of voucher specimen's stems as provided by the Missouri Botanical Garden (St. Louis, MO). The same information can be found at http://www.mobot.org/MOBOT/research/diversity/herbarium/compendium_model.aspx?id=3.

A temporary water mount of SRM 3240 was prepared by mixing the dry powder with water and waiting until the material was fully hydrated. SRM 3240 consists of a mixture of different size particles of two major types. Small particles are pieces of individual cells that are not generally identifiable to cell type, angular but not sharply so, 0.01 mm to 0.07 mm in diameter, translucent and nearly colorless to light tan. The larger particles are tan to golden-brown, multi-cellular, 0.5 mm to 0.7 mm × 0.3mm to 0.5 mm, consisting of epidermal cells and inner parenchyma, the parenchyma cells columnar or rectangular, parallel-sided or angular, 0.1 mm to 0.15 mm × 0.03 mm to 0.05 mm.

Stems of *E. sinica* (voucher specimen *Zhang* 1793) are mostly 1 mm to 2 mm in diameter. In cross-section, they appear roughly round, with prominent, usually rounded ridges. Epidermal cells are rounded and thick-walled, mostly 12 µm to 21 µm (but rarely to 27 µm) in diameter. They secrete an acellular waxy cuticle, 9 µm to 15 µm (but rarely to 24 µm) thick, which has a slightly layered appearance and contains numerous tiny dark inclusions. Stomata may occur in the valleys between the ridges, most noticeably as gaps in the epidermis and cuticle. Each ridge contains a fiber bundle which begins immediately beneath the epidermis and occupies at least the central portion of the ridge, often filling the entire apical portion of the ridge. Fibers are rounded-polygonal, 9 µm to 23 µm in diameter, slightly smaller and less deeply staining than the epidermal cells; the walls are thickened but not lignified. Beneath the epidermis and ridges, there is a fairly thick ring of cortical parenchyma (within which small bundles of fibers may sometimes be found). Cortical parenchyma cells are thin-walled, rounded- to rectangular-polygonal with the longest dimension oriented radially, typically to 40 µm in length. The vascular cambium is roughly circular; vascular bundles are somewhat top-shaped and eight to ten in number. Outside each phloem bundle, there is a small crescent-shaped to flattened triangular bundle of thick-walled, nonlignified fiber cells similar in appearance to those in the ridges. Phloem bundles are flattened, usually somewhat lenticular in shape with both outer and inner edges convex; phloem cells are arranged in ranks, small (to 6 µm to 10 µm long) and thin-walled. Xylem bundles are triangular, frequently concave at the cambial edge, containing thick-walled,

lignified tracheids arranged in distinct ranks; individual cells are rectangular, mostly 4.5 µm to 15 µm across, often somewhat irregularly shaped with curved long walls, with those near the cambium being smallest and most flattened; at the point of each xylem bundle, there may be a few rounded to elliptical cells, to 24 µm long, extending into the pith. The pith is composed of large, roughly rounded to elliptical thin-walled parenchymatous cells, up to 120 µm in the longest direction, which have small triangular extracellular spaces at their junctures; pith cells sometimes have dark-staining contents.