National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 3243

Ephedra-Containing Solid Oral Dosage Form

This Standard Reference Material (SRM) 3243 is intended primarily for use in validating analytical methods for the determination of ephedrine alkaloids, caffeine, synephrine, and toxic elements in ephedra-containing dietary supplements, particularly solid oral dosage forms, and similar matrices. This SRM can also be used for quality assurance when assigning values to in-house control materials. SRM 3243 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 3243 consists of ten bottles of powdered solid oral dosage form, each containing approximately 2.5 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 by NIST [1]. The certified concentration values of selected ephedrine alkaloids, synephrine, caffeine, 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 non-certified value that is the best estimate of the true value; however, the value does not meet NIST criteria for certification [1] and is provided with associated uncertainties that may reflect only measurement precision and may not include all sources of uncertainty. 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 3243 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.

Acquisition and preparation of the material was coordinated by K.E. Sharpless of the NIST Analytical Chemistry Division. Material preparation was performed by B.J. Porter of the NIST Analytical Chemistry Division.

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Analytical measurements at NIST were performed by J.M. Brown Thomas, 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, 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 CFSAN (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.

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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 material is exempt from requirements of Title 21 Code of Federal Regulations Section 1310 (21 CFR 1310). Concentrations of ephedrine alkaloids in this material are less than those specified in section 1310.12 (c) of the regulation, thereby exempting this material.

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 in the "Preparation and Analysis" section were 0.5 g and 1 g for ephedrine alkaloids, 1 g for synephrine, 0.15 g and 1 g for caffeine, 0.2 g for arsenic, 0.25 g for mercury, and 1 g for cadmium and lead.

PREPARATION AND ANALYSIS[1]

Material Acquisition and Preparation

SRM 3243 was prepared from several different commercially available products (both tablets and capsules) that were purchased in the marketplace. The products were intentionally purchased from multiple vendors to obtain material from different production lots. The tablets and the contents of the capsules were ground using a Teflon disc mill at room temperature, and the powdered material was then sieved to 177 μm (80 mesh). The powdered material was shipped to Sun-Ten (Irvine, CA) where it was blended for 20 min in a V-blender. The material was subsequently transferred to ChromaDex, Inc. (Santa Ana, CA) where it was 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 12.5 kGy to 15.7 kGy.

Analytical Approach for Determination of Ephedrine Alkaloids, Synephrine, and Caffeine

Value assignment of the concentrations of the ephedrine alkaloids in SRM 3243 was based on the combination of measurements from different analytical methods at NIST and at three collaborating laboratories. As many as 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

[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.

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 3243. The analytical methods for the ephedrine alkaloids used by NIST and the collaborating laboratories are described in detail in reference 8.

Value assignment of the concentrations of synephrine and caffeine in SRM 3243 was based on the combination of measurements from different analytical methods at NIST and at two collaborating laboratories. Synephrine was determined at NIST by using LC/MS/MS and LC/MS, at FDA by using LC/MS/MS, and at ChromaDex by using LC/UV. Caffeine was determined at NIST by using LC/UV and LC/MS/MS and at Chromadex by using LC/UV.

NIST Analyses for Ephedrine Alkaloids and Synephrine

Ephedrine alkaloids and synephrine 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 filtered 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, 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 utilized 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 °C ± 0.5 °C 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 A.

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 152 (norephedrine and norpseudoephedrine), 166 (ephedrine and pseudoephedrine), 180 (methylephedrine and methylpseudoephedrine), and 169 (ephedrine-d_3). Synephrine was also determined by using this method based on monitoring the ion (*m/z*) at 150. Ephedrine-d_3 was used as the internal standard for LC/MS measurements. A typical separation is provided in Appendix A.

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 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 A. Synephrine was also determined using this method based on precursor and fragment ions (*m/z*) 167.9 and 150.

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 A; note that only the (–)-ephedrine and (+)-pseudoephedrine enantiomers that are naturally occurring in *E. sinica* were found in this material, indicating that the material was not altered through the addition of synthetic alkaloids.

NIST Analyses for Caffeine

Caffeine was determined at NIST by using LC/UV and LC/MS/MS. For the LC/UV analyses, duplicate subsamples of approximately 150 mg were removed from each of six bottles of SRM 3243. Approximately 7 g of a methanol solution of β-hydroxyethyltheophylline was added to the subsample for use as an internal standard; the methanol served as the extraction solvent. After sonication for 1 h, the samples were processed as described above and analyzed by using reversed-phase LC/UV on a C_{18} column as described in reference 10. The LC/MS/MS measurements of caffeine were obtained during the LC/MS/MS analyses for the ephedrine alkaloids described above. $^{13}C_3$-caffeine was added to the subsamples of SRM 3243 for use as an internal standard in the LC/MS/MS analyses.

Analytical Approach for Determination of Elements

The elements of primary interest in SRM 3243 were the potentially toxic contaminants arsenic, cadmium, lead, and mercury. Value assignment of the concentrations of toxic elements in SRM 3243 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 lead, and cold vapor (CV) ID ICP-MS was used for determination of mercury. NIST also provided measurements of chromium by using inductively coupled plasma atomic emission spectrometry (ICP-AES). 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, phosphorus, potassium, samarium, silicon, sodium, sulfur, and zinc. All collaborating laboratories analyzed a minimum of six subsamples (one from each of six bottles or two from each of three bottles) of SRM 3243.

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} $cm^{-2} \cdot s^{-1}$ 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 and lead determinations, a single 1 g portion was taken from each of six bottles of the SRM. Isotopically enriched ^{111}Cd and ^{206}Pb were added to the samples prior to digestion in Teflon beakers by wet ashing with nitric acid, hydrofluoric acid, and perchloric acid at 185 °C for 24 h. The residue was redissolved in 2 % nitric acid, and measurements were made by using ID ICP-MS [11].

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 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 [12].

For chromium determinations, duplicate subsamples of 0.5 g from each of six bottles of the SRM were prepared by microwave-assisted digestion with nitric acid. Indium was added to each sample as an internal standard, and the concentration of chromium was determined by the method of standard additions by using ICP-AES.

NIST Determination of Moisture

Moisture content of SRM 3243 was determined by (1) freeze-drying to constant mass over 7 days; (2) drying over magnesium perchlorate in a desiccator at room temperature for 55 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 0.9537 (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 and caffeine was assessed at NIST by using the LC/UV methods described above. An analysis of variance using measurements for ephedrine did not show inhomogeneity for a 1 g sample. An analysis of variance on results for caffeine did show a mean bottle difference of 1.6 % for 150 mg samples, and an inhomogeneity component has been included in the expanded uncertainty for the caffeine. Other measurands were treated as though they were homogeneous, although homogeneity was not assessed.

Value Assignment

The equally weighted means from each set of data were used to calculate the assigned values unless otherwise stated in the tables. 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. In the case of chromium, for which only NIST made measurements, the mean of the NIST results became the assigned value.

Table 1. Certified Concentration Values for Ephedrine Alkaloids, Synephrine, and Caffeine in SRM 3243[a]

Analyte	Mass Fraction (mg/g)
Ephedrine[b,c,d,e,f,g,h,i,j]	11.21 ± 0.42
Methylephedrine[b,c,d,f,g,i,j]	0.323 ± 0.031
Pseudoephedrine[b,c,d,e,f,g,h,i,j]	2.81 ± 0.11
Total Alkaloids[b,c,d,f,g,i,j]	14.78 ± 0.54
Synephrine[c,d,f,g]	0.54 ± 0.19
Caffeine[b,d,g,k]	76.5 ± 4.1

[a] Each certified concentration value, expressed as a mass fraction on a dry-mass basis, is an equally weighted mean of the results from three to nine analytical methods carried out at NIST and at collaborating laboratories. The uncertainty in each 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. (See footnote for caffeine.) 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
[k] Expanded uncertainty includes a contribution of 1.6 % due to inhomogeneity.

Table 2. Certified Concentration Values for Selected Elements in SRM 3243[a]

Element	Mass Fraction (mg/kg)
Arsenic[b,c]	0.554 ± 0.018
Cadmium[d,e,f]	0.1218 ± 0.0033
Lead[d,e,f]	0.692 ± 0.056
Mercury[g]	0.00900 ± 0.00044

[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 each 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 INAA
[c] NRCC HG-GFAAS
[d] NIST ID ICP-MS
[e] NRCC ID ICP-MS
[f] FDA ICP-MS
[g] NIST CV ID ICP-MS

Table 3. Reference Concentration Values for Ephedrine Alkaloids in SRM 3243[a]

Analyte	Mass Fraction (mg/g)
Methylpseudoephedrine[b,c,d,e,f]	0.020 ± 0.011
Norephedrine[b,c,d,e,f,g,h,i]	0.160 ± 0.026
Norpseudoephedrine[b,c,d,e,f,g,h,i]	0.186 ± 0.029

[a] Each reference concentration value, expressed as a mass fraction on a dry-mass basis, is an equally weighted mean of the results from five to eight analytical methods carried out at NIST and at collaborating laboratories. The uncertainty in each 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] NRCC LC/UV
[f] NRCC LC/MS/MS
[g] FDA LC/MS/MS
[h] ChromaDex LC/UV
[i] NRCC FAIMS

Table 4. Reference Concentration Values for Selected Elements in SRM 3243[a]

Element	Mass Fraction (%)
Calcium	1.03 ± 0.05
Carbon	38.5 ± 1.1
Chlorine	1.07 ± 0.03
Hydrogen	5.32 ± 0.10
Magnesium	4.80 ± 0.14
Nitrogen	4.45 ± 0.21
Potassium	1.39 ± 0.03
Silicon	1.62 ± 0.03

Element	Mass Fraction (mg/kg)
Boron	70.6 ± 1.4
Chromium[b]	63.4 ± 1.3
Gadolinium	0.133 ± 0.007
Iron	760 ± 160
Phosphorus	6800 ± 1000
Samarium	0.132 ± 0.009
Sodium	1960 ± 140
Sulfur	2630 ± 100
Zinc	3250 ± 310

[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. (See separate footnote for chromium.) The uncertainty in each 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.
[b] The reference concentration value for chromium, expressed as a mass fraction on a dry-mass basis, is the equally weighted mean of values provided by NIST using ICP-AES. 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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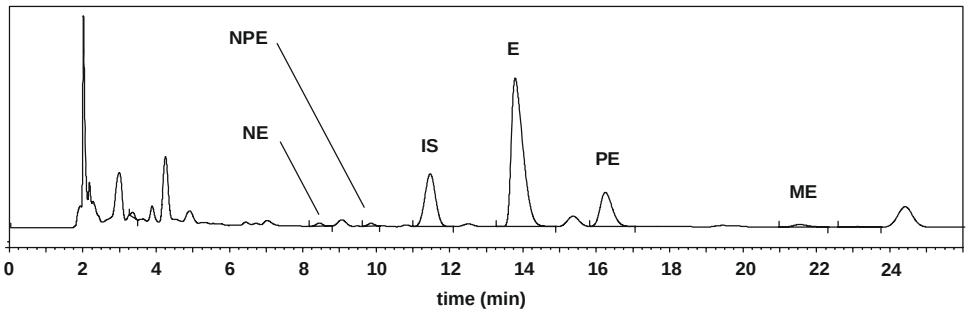
Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-6776; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.

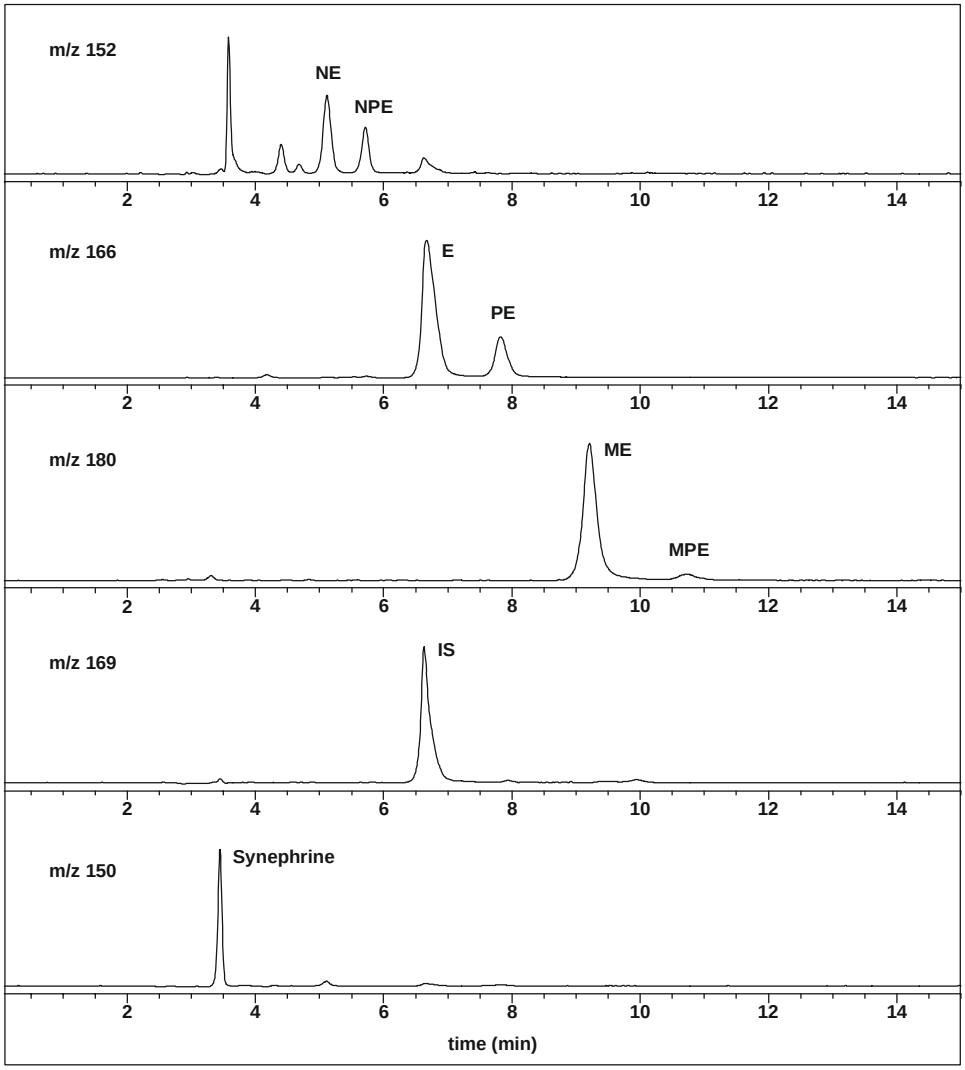
APPENDIX A

Typical chromatograms from the analysis of SRM 3243 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].

a)

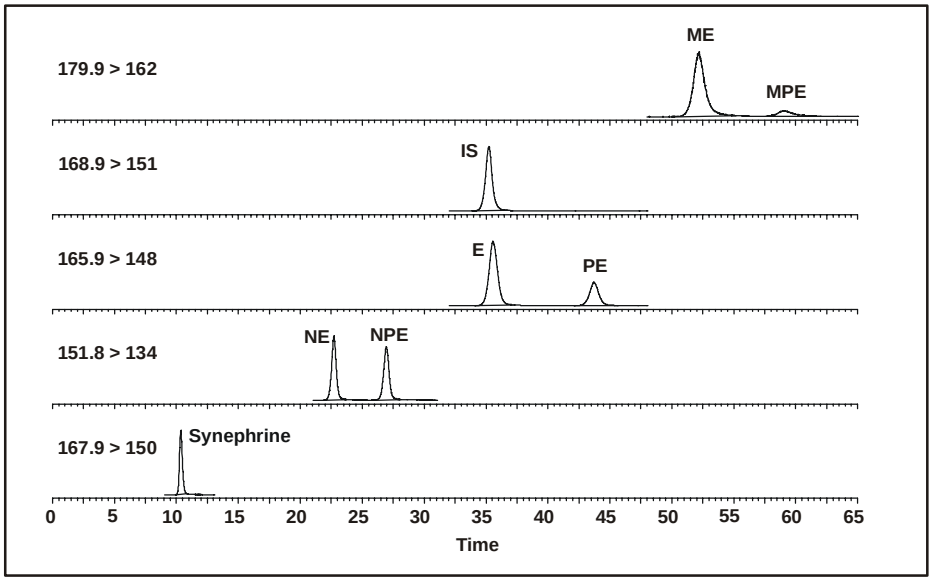


b)

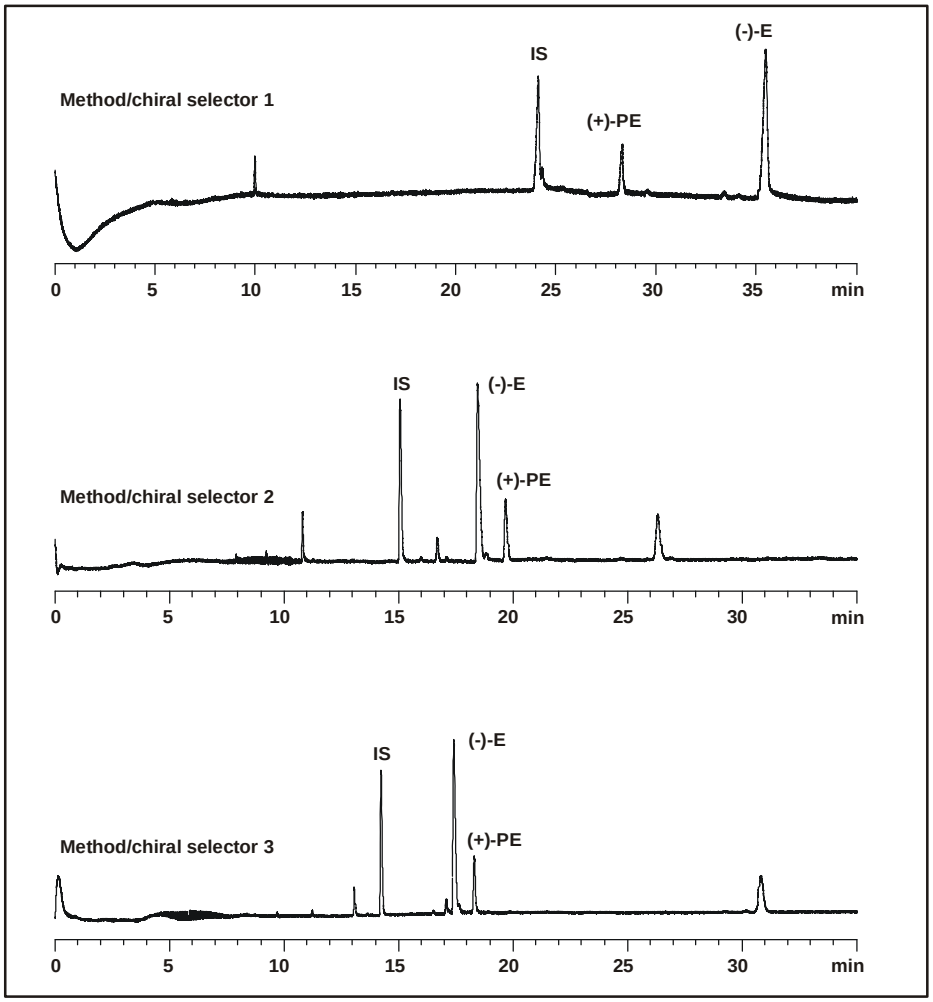


Typical chromatograms from the analysis of SRM 3243 by using a) LC/UV; b) LC/MS; c) LC/MS/MS; d) CE.

c)



d)



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Please complete and return this registration card to the address given on the reverse side.

This will register your SRM so you can be notified of any updates or developments.
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Thank you.

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