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Determination of Ephedrine Alkaloids in Dietary Supplement Standard Reference Materials

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A suite of five ephedra-containing dietary supplement Standard Reference Materials (SRMs) has been issued by the National Institute of Standards and Technology (NIST) with certified values for ephedrine alkaloids, synephrine, caffeine, and selected toxic trace elements. The materials represent a variety of natural, extracted, and processed sample matrixes that provide different analytical challenges. The constituents have been determined by multiple independent methods with measurements performed by NIST and by three collaborating laboratories. The methods utilized different sample extraction and cleanup steps in addition to different instrumental analytical techniques and approaches to quantification. In addition, food−matrix proximates were determined by National Food Processor Association laboratories for one of the ephedra-containing SRMs. The SRMs are primarily intended for method validation and for use as control materials to support the analysis of dietary supplements and related botanical materials.

The enactment of the Dietary Supplement Health and Education Act (DSHEA) in 1994[1] by the U. S. Congress has promoted growth in the nutritional supplement industry, due in part to the way in which dietary supplements are regulated. DSHEA provides a legal definition of dietary supplements that classifies these materials separately from food additives and pharmaceutical drugs. Requirements for product labeling are less stringent than for drug substances, and the burden of proof for the safety of dietary supplements is placed on the Food and Drug Administration (FDA).

The powdered botanical *Ephedrae herba* (Ma-Huang, or simply "ephedra") is widely used in traditional Chinese medicine to induce perspiration, reduce fever, and treat coughs and asthma.[2] Ephedra-containing dietary supplements have been promoted for use as an aid in dieting and as a stimulant for boosting energy and athletic performance. The activity of ephedra is attributed to the presence of three pairs of diastereomeric ephedrine alkaloids: (1*R*,2*S*)-(−)-norephedrine and (1*S*,2*S*)-(+)-norpseudoephedrine, and (1*R*,2*S*)-(−)-ephedrine, and (1*S*,2*S*)-(+)-pseudoephedrine, (1*R*,2*S*)-(−)-*N*-methylephedrine, and (1*S*,2*S*)-(+)-*N*-methylpseudoephedrine (see Figure 1), of which ephedrine constitutes the largest fraction. Over 50 species of ephedra are known worldwide, and at least 18 species contain significant levels of these alkaloids.[3−5] Only one enantiomer of each of these alkaloids is present in natural sources, although the complementary enantiomers have been prepared synthetically. Levels of these alkaloids vary with plant species, time of harvest, geographical location, and growing conditions. These factors, combined with lack of regulatory requirements for quality control, fostered an environment in which product variability, potential adulteration, and/or contamination could have represented a public health risk.

In December 2003, the FDA issued a ruling that declared dietary supplements that contain ephedrine alkaloids to be adulterated.[6] This ruling was based on mounting evidence of

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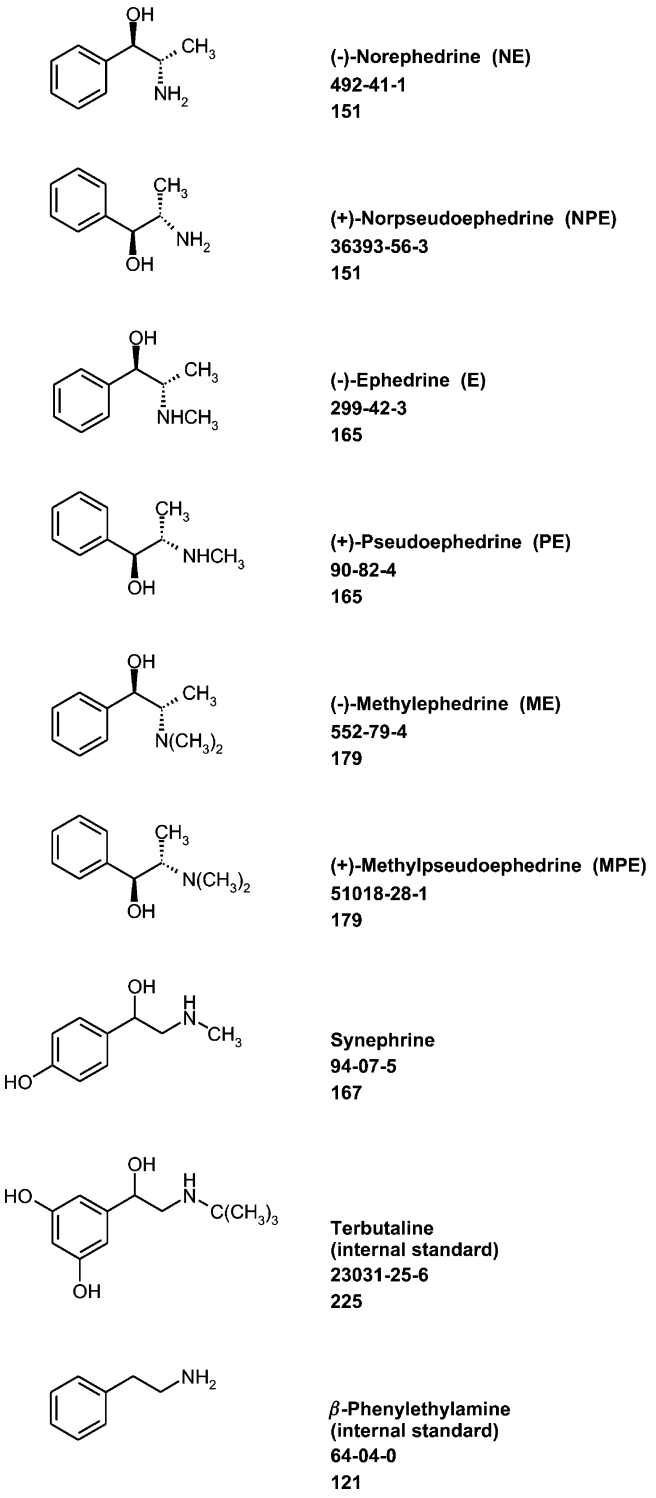

Figure 1. Structures of ephedrine alkaloids and related compounds, including CAS designations and relative molecular masses.

health risks associated with the use of ephedra and in effect bans the use of ephedrine alkaloids (regardless of their botanical origin) in dietary supplements. The National Institute of Standards and Technology (NIST), working in collaboration with the National Institutes of Health Office of Dietary Supplements (NIH-ODS) and FDA, Center for Drug Evaluation and Research (CDER) and Center for Food Safety and Applied Nutrition (CFSAN), has recently issued a suite of Standard Reference Materials (SRMs) that contain ephedra. Five SRMs are available: SRM 3240 *Ephedra sinica* Stapf Aerial Parts, SRM 3241 *Ephedra sinica* Stapf Native Extract, SRM 3242 *Ephedra sinica* Stapf Commercial Extract, SRM 3243 Ephedra-Containing Solid Oral Dosage Form, and SRM 3244 Ephedra-Containing Protein Powder. In addition, SRM 3245 is available and contains two bottles each of the five ephedra-containing materials. The SRMs are certified for levels of ephedrine alkaloids and selected toxic trace elements. In addition, the level of synephrine (an ephedrine-like alkaloid present in many ephedra-free dietary supplements) is certified in SRM 3243, and levels of caffeine are certified in SRM 3243 and SRM 3244. Information on proximates (i.e., moisture, solids, ash, protein, carbohydrates, fat) and nutrient elements is also included with SRM 3244. These SRMs are intended for use in method validation and as control materials for analytical methods used in the determination of ephedrine alkaloids and should prove to be useful to support such methods and to demonstrate the absence of ephedrine alkaloids in ephedra-free products.

The ephedra-containing SRMs represent a variety of natural, extracted, and processed sample matrixes, and as such the materials provide different analytical challenges. Value assignment of alkaloid content was approached through application of multiple analytical methods, which included measurements by NIST and measurements by collaborating laboratories. Different sample processing methods, instrumental analytical techniques, and approaches to quantification were utilized to provide measurement independence. This paper details the analysis of five ephedra-containing SRMs for alkaloid content by using nine analytical approaches and the subsequent value assignment.

EXPERIMENTAL SECTION

[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.]

SRM Preparation. A cultivated crop of *Ephedra sinica* Stapf was harvested in 2002 and processed resulting in a dried, powdered material that was used to prepare SRM 3240 *E. sinica* Stapf Aerial Parts. Portions of this material were further processed to prepare extracts at a "native" level (~4% mass fraction total alkaloids) and a "commercial" level (~8% mass fraction total alkaloids). SRM 3243 Ephedra-Containing Solid Oral Dosage Form was prepared by grinding and blending commercially available dietary supplements (both tablets and the contents of capsules) and SRM 3244 Ephedra-Containing Protein Powder is a similar blend of commercially available protein drink mixes.

Reagents (NIST). Unless indicated otherwise, ephedrine alkaloid reference standards were obtained from ChromaDex (Santa Ana, CA). Norpseudoephedrine (cathine, a schedule IV controlled substance) and synephrine were obtained from Sigma (St. Louis, MO). Ephedrine-d_3 was obtained from Isotec (Miamisburg, OH). The purities of reference standards were determined from a consensus of multiple methods at NIST, including differential scanning calorimetry, liquid chromatography with ultraviolet absorbance detection (LC/UV), liquid chromatography with mass spectrometric detection (LC/MS), and manufacturer data. Purities for all reagents were better than 98% (mass fraction, except norpseudoephedrine hydrochloride, which was 97.3% by

mass fraction). The moisture content of the SRMs was also determined from a consensus of multiple methods including drying in a desiccator over magnesium perchlorate, drying in a forced air oven at 85 °C for 4 h, and lyophilization over the course of 7 or 11 d. Data are reported on a dry-mass basis using individual drying factors for each of the five materials. Sources of reference standards for collaborating laboratories are listed separately in the descriptions of analytical methods.

Method 1: LC/UV (NIST). Six samples of each SRM were weighed into 50-mL polyethylene centrifuge tubes or glass pressurized fluid extraction tubes, followed by the addition of a measured mass of internal standard solution (terbutaline). Sample amounts were adjusted depending on the levels of alkaloids present and ranged from ~0.15 g for SRM 3242 *Ephedra sinica* Stapf Commercial Extract to ~5 g for SRM 3244 Ephedra-Containing Protein Powder. Approximately 30 g of methanol was added to each of 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. During this time, bath temperature gradually increased to ~50 °C. At the completion of the sonication extraction, the suspended solids 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. Injection volume was 10 μL for most samples, and 2 μL for calibrants, to provide similar absolute responses. An isocratic LC method 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. A 0.1 mol/L KH_2PO_4 solution was prepared in a ~3.5% volume fraction solution of methanol in water, for use as the mobile phase. Column temperature was controlled at 29 ± 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.

Method 2: LC/MS (NIST). Ten samples of each SRM were weighed into glass-frit Soxhlet thimbles each containing an ~1-cm layer of diatomaceous earth (Hydromatrix, Isco, Lincoln, NE). After stirring, additional diatomaceous earth was added (~1 cm). A measured mass of internal standard solution (ephedrine-d_3) was transferred to each Soxhlet thimble. The samples were Soxhlet extracted with 200 mL of methanol for at least 18 h. Extracts were concentrated to ~1 mL under nitrogen, and the sides of the tube were rinsed with methanol to yield a final volume of 10 mL. This extract was filtered through a 0.45 μm × 2.5 cm syringe filter. An isocratic LC/MS method similar to the method of Gay et al. [8] was used for the determination of ephedrine alkaloids and synephrine. A 250 mm × 4.6 mm YMC Phenyl column (Waters, Inc., Milford, MA) was used at ambient temperature (21 ± 1 °C) with an isocratic mobile phase with a flow rate of 1.0 mL/min. The mobile phase consisted of a solution containing approximately 2% methanol, 2% glacial acetic acid, and 0.4% ammonium acetate in water (mass fractions). For analyses of SRM 3243 and SRM 3244, ammonium acetate was omitted. This reduced analysis time; however, the absolute retention of the alkaloids was found to be less reproducible with new mobile-phase preparations, and the acetate buffer was utilized for SRMs 3240, 3241, and 3242. The mass spectrometer was operated in positive ion mode, with atmospheric pressure ionization electrospray ionization (API-ES). Sample injections of 1 μL were made. Quantification of the seven alkaloids was based on signals monitored at a mass-to-charge ratio (m/z) of 150 (synephrine), 134 and/or 152 (norephedrine and norpseudoephedrine), 148 or 166 (ephedrine and pseudoephedrine), 180 (methylephedrine and methylpseudoephedrine), and 169 (ephedrine-d_3).

Method 3: LC/MS/MS (NIST). Six samples were prepared as with method 1, except the sonication extraction was carried out for 30 min. Chromatographic conditions were similar to those used in method 2; however, the flow rate was reduced to 0.5 mL/min and column temperature was set at 30 ± 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 were monitored in the third quadrupole. The following precursor and fragment ions were monitored (m/z): 168 → 150 (synephrine), 152 → 134 (norephedrine and norpseudoephedrine), 166 → 148 (ephedrine and pseudoephedrine), 180 → 162 (methylephedrine and methylpseudoephedrine), and 169 → 151 (ephedrine-d_3).

Method 4: CE (NIST). Six samples of each SRM were weighed into 50-mL polyethylene centrifuge tubes, followed by the addition of measured masses of internal standard solution (β-phenylethylamine hydrochloride) and ~18 mL of methanol. Sample sizes were 0.5 g for SRM 3240, 0.25 g for SRM 3241, 0.1 g for SRM 3242, 0.5 g for SRM 3243, and 2.5 g for SRM 3244. The samples were sonicated for 30 min, and the supernatant solution was filtered through 0.2-μm nylon 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, and details are described elsewhere.[9] The methods were sufficiently dissimilar 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 15−30 kV.

Method 5: LC/MS/MS (FDA). Three samples of each SRM were weighed into 50-mL polyethylene centrifuge tubes followed by the addition of the internal standard (1 mg/mL of ephedrine-d_5 in extraction solvent, 0.4 mL). Samples sizes were adjusted depending on the levels of alkaloids present; approximate amounts were as follows: SRM 3240, 100 mg; SRM 3241, 40 mg; SRM 3242, 15 mg; SRM 3243, 100 mg; SRM 3244, 1000 mg. Samples were extracted with 20-mL aliquots of methanol/water (80:20 volume fraction) by sonication for 30 min. The samples were then centrifuged at 942 rad/s (9000 rpm) for 20 min, and the extracts were analyzed without solid-phase extraction (SPE) cleanup. An isocratic LC method was used with a YMC Phenyl, 2 mm × 250 mm, 5-μm particle size column (Waters). The isocratic method used a mobile-phase composition of 1% (volume fraction) acetonitrile, 2% (volume fraction) acetic acid, and 97% (volume fraction)

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50 mmol/L ammonium acetate in water. Detection was with a triple quadrupole mass spectrometer with atmospheric pressure chemical ionization source operated in the MS/MS mode. Three MRM transitions were monitored for each precursor ion for unambiguous component identification (m/z): 168 → 118, 135, 150 (synephrine), 152 → 117, 134, 152 (norephedrine and norpseudoephedrine), 166 → 117, 133, 148 (ephedrine and pseudoephedrine), 180 → 134, 147, 162 (methylephedrine and methylpseudoephedrine), and 171 → 121, 138, 153 (ephedrine-d_5). Quantification was based on the sum of the ion currents for each transition.

Method 6: LC/UV (ChromaDex). The method of AOAC International Collaborative Study Protocol Ka012 was followed for all measurements, except results are based on a single-point calibration curve.[7] Primary standards were from ChromaDex, except norpseudoephedrine (Cerilliant Corp., Round Rock, TX), ephedrine hydrochloride (Aldrich, Milwaukee, WI), and pseudoephedrine hydrochloride (Sigma). Samples of SRM 3244 were prepared slightly differently from SRMs 3240−3243. Six 10-g samples of SRM 3244 were weighed into 100-mL volumetric flasks. Samples were extracted with ~40 mL of methanol by shaking for 15 min, followed by sonication for 45 min. One milliliter of 500 mmol/L H_3PO_4 was added; the solutions were diluted to volume, centrifuged, and a portion of the extract was processed by SPE. Samples sizes for the other SRMs were adjusted depending on the levels of alkaloids present: ~0.2 g for SRMs 3241 and 3242 and ~1.5 g for SRMs 3240 and 3243. Samples were extracted with ~50 mL of a diluent consisting of 3% methanol and 97% of 10 mM potassium phosphate monobasic buffer by shaking for 15 min, followed by sonication for 45 min. The solutions were diluted to 100 mL with the diluent, centrifuged, and a portion of the extract was processed by SPE. A Strata SCX 500 mg × 3 mL SPE cartridge (Phenomenex) was conditioned sequentially with 2 mL of methanol and 1 mL of 50 mmol/L H_3PO_4. Five-milliliter aliquots were loaded onto the SPE, washed with 1 mL of 50 mmol/L H_3PO_4 and 2 mL of methanol, and eluted with three 1-mL aliquots of NH_4OH/methanol (5:95 volume fraction). The eluates were diluted to 10.0 mL with 500 mmol/L H_3PO_4 and analyzed by LC/UV. An isocratic LC method was used with a 4.6 mm × 150 mm Synergi Polar-RP column (Phenomenex) operated at 25 °C. The mobile phase was 3:97 (volume fraction) methanol/0.1 mmol/L KH_2PO_4, at 1.5 mL/min flow rate. Detection was at 210 nm, and 20-μL injections were made.

Separate analyses were carried out for the determination of synephrine in SRM 3243. A different LC method, which employed ion pairing, was used to resolve synephrine from matrix interferences. Approximately 1-g samples of SRM 3243 were extracted in water by shaking for 15 min, followed by sonication for 15 min. LC/UV analyses were carried out with a 4.6 mm × 150 mm Luna C18(2) column (Phenomenex) operated at 25 °C, with a mobile phase consisting of 35:65:0.1 acetonitrile/water/H_3PO_4 containing 10 mmol/L sodium dodecyl sulfate and a flow rate of 1.5 mL/min. Detection was at 220 nm, and 20-μL injections were made.

Method 7: High-Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS; National Research Council of Canada, NRCC). Six samples of SRMs 3240 and 3243 were analyzed by an internal standard method. The mass of material used in each extraction was adjusted to achieve reasonable analyte concentrations in the final extract. For SRM 3240, 1 g of material was extracted, and for SRM 3243, 0.5 g was extracted. The candidate material was weighed directly into 50-mL volumetric flasks and samples were extracted with ~25 mL of 500 mmol/L ammonium formate in methanol/water (3:97 volume fraction) by shaking for at least 15 min and sonication for at least 45 min. After cooling, the samples were diluted to 50 mL with the extractant and shaken well. An aliquot of the extract was removed and centrifuged to remove the suspended solids, and a portion of the supernatant was filtered through a 0.45 μm × 2.5 cm syringe filter. An aliquot of the filtered sample was weighed and spiked with deuterated ephedrine hydrochloride. This mixture was diluted 200−500-fold with methanol/water (95:5 volume fraction) containing 0.1 mmol/L ammonium acetate. Analytical measurements were made using flow injection ESI-FAIMS-MS. A spiked sample (70 μL) was injected into a running buffer (90:10 volume fraction methanol/water containing 0.2 mmol/L ammonium acetate). The stream was split about 100 to 1 and delivered to the electrospray needle. The FAIMS device was operated in P1 mode, i.e., positive ion polarity and positive dispersion voltage, with 2.5 L/min nitrogen curtain/carrier gas for ephedrine, pseudoephedrine, norephedrine, and norpseudoephedrine and in P2, i.e., positive ion polarity and negative dispersion voltage, with 2.5 L/min nitrogen/helium (60:40) curtain/carrier gas for methylephedrine. The optimum compensation voltages for transmission of each target analyte were −5.2 V for ephedrine (m/z = 166), −6.9 V for pseudoephedrine (m/z = 166), −6.0 V for norephedrine (m/z = 152), −7.8 V for norpseudoephedrine (m/z = 152), and −6.0 V (P2) for methylephedrine (m/z = 180). The CV of the internal standard, deuterated ephedrine (m/z = 169), was −4.7 in P1 mode and −2.5 in P2 mode. The mass spectrometer was operated in SIM mode monitoring the M + H ion for each analyte and the internal standard.

Method 8: LC/UV (NRCC). At least six samples of SRMs 3240, 3241, 3243, and 3244 were analyzed by a standard addition method (without internal standard). The mass of material used in each extraction was adjusted to achieve reasonable analyte concentrations in the extract: 1 g of material for SRM 3240, 0.25 g for SRM 3241, 0.5 g for SRM 3243, and 5 g for SRM 3244. A standard addition calibration approach was used for quantitation. Samples were prepared in duplicate, and a known amount of a matched pure standard mixture was added to one of the pairs of samples. The mixture was equilibrated overnight at room temperature. The extraction method is the same as in method 7 except for SRM 3244, for which material was prepared directly into 100-mL volumetric flasks and ~40 mL of 500 mM ammonium formate extractant was used in the original sonication step. After cooling, the samples were diluted to volume and shaken well. A portion of the extract was centrifuged and cleaned-up by a Strata SCX SPE cartridge (Phenomenex). The cartridges were conditioned by successive washings with 2 mL of methanol followed by 1 mL of 50 mM phosphoric acid. Each sample extract (5−10 mL) was applied to an SPE cartridge by gravity elution, after which the cartridge was washed with 1 mL of 50 mmol/L phosphoric acid, followed by 2 mL of methanol. The cartridge was allowed to dry between each step. Finally, the targeted analytes were eluted from the cartridge with three successive 1-mL volumes of SPE elution solvent into a 10-mL volumetric flask. The collected eluate was

acidified and diluted to volume with 500 mmol/L phosphoric acid. This working sample solution was thoroughly mixed by vortexing, and a portion was transferred to a HPLC sample vial for LC/UV analysis. An Agilent Technologies (Palo Alto, CA) 1100 LC system equipped with diode array detector and an autosampler was used. A Phenomenex Synergi Polar-RP column (4.6 mm × 150 mm) packed with 4-μm particles and held at 25 °C was used throughout. Injection volumes of 20 μL were used, and analytes were eluted isocratically with 100 mmol/L potassium phosphate in 3% methanol (mobile phase) at 1.75 mL/min for the first 16 min. The column was then flushed with acetonitrile/water/phosphoric acid (50:50:0.1) for 6 min to remove the more nonpolar type components, such as caffeine, which might have been coextracted with the analytes during sample preparation. Finally, the column was equilibrated with the mobile phase for 10 min prior to the next injection. The diode array detector was set to monitor at 210 nm (bandwidth 8 nm), and analyte peaks from each chromatogram were manually integrated and the data exported to Excel for further processing. All calculations, except for the HPLC injections, were based on gravimetric measurements recorded as mass.

Method 9: LC/MS/MS (NRCC). Samples of SRMs 3240, 3241, 3243, and 3244 were analyzed by a standard addition method (without an internal standard). From each bottle, paired samples were prepared using 0.25−5-g portions, and the samples were extracted as in method 8. Aliquots of the raw extract (~1 mL) were cleaned according to the protocol described by Trujillo and Sorenson.[10] Measurements were made using LC/MS/MS with ES ionization. An isocratic LC method was used with a 2.0 mm × 250 mm YMC phenyl S-3 column. The mobile phase was 95:3:2 (volume fraction) water/acetonitrile/acetic acid containing 50 mmol/L ammonium acetate. The flow rate was 0.23 mL/min, and injection volume was 10 mL. The mass spectrometer was operated in MRM mode monitoring transitions (m/z) 152 ± 134 (norephedrine and norpseudoephedrine), 166 ± 148 (ephedrine and pseudoephedrine), and 180 ± 162 (methylephedrine and methylpseudoephedrine). A standard additions scheme was used for quantification.

RESULTS AND DISCUSSION

SRMs 3240−3244 represent the first in a series of dietary supplement reference materials under development by NIST. The ephedra-containing Standard Reference Material suite was developed in collaboration with NIH (ODS) and FDA (CDER and CFSAN) to meet a need for matrix-based reference materials for use in method validation and as control materials for analytical methods used in the determination of ephedrine alkaloids. The five materials span a range of matrix types that are of significance in the dietary supplement industry. In the formulation of dietary supplements, it is common for manufacturers to use extracted forms of a plant rather than the less processed dried plant. Here, the term "extract" refers to a powdered solid consisting of an excipient upon which the extracted constituents are deposited. Commercial extracts are often normalized to specific levels of "marker compounds" to improve product consistency and, in certain cases, to increase levels of active constituents. The marker compounds are intended to be representative of a specific herbal material, but are not necessarily the active constituents, since the origin of herbal activity may not be known or may result from a combined effect of several constituents.

The process of assigning values for chemical composition in reference materials at NIST has recently been described in detail.[11] Three types of values may be reported in SRMs: certified values, reference values, and information values. Certified values are values upon which NIST places the highest level of confidence in that known or suspected sources of bias have been investigated or accounted for. The most common approach for certification of chemical composition is measurement by two or more independent analytical methods. The use of independent methods (including but not limited to extraction, cleanup, instrumental analysis, and approach to quantification) should provide indications of method accuracy, since biases would result in disagreement among methods. Measurements that do not meet the criteria required for certification are reported as reference values or information values.[11]

Value assignment of the concentrations of the ephedrine alkaloids in the five ephedra-containing SRMs was based on the combination of measurements from different analytical methods at NIST and at three collaborating laboratories. These measurements exceed the requirements for certification of natural-matrix SRMs; however, a concerted effort was made for this first botanical dietary supplement SRM to include different stakeholders in the measurement community in the value assignment process: FDA is a regulatory agency with interest and expertise in the analysis of dietary supplements, NRCC is the National Metrology Institute (NMI) of Canada and also supplies Certified Reference Materials (NIST is the NMI of the USA), and ChromaDex is a private-sector manufacturer that specializes in chemical standards related to botanicals.

A total of nine sets of measurements were used for the value assignment of the concentrations of ephedrine alkaloids (see Figure 2). NIST provided measurements using a combination of two sample extraction procedures and three LC methods with different modes of detection, i.e., UV absorbance, MS, and MS/MS. Chiral measurements of (−)-ephedrine and (+)-pseudoephedrine were carried out by CE. Results for ephedrine alkaloids were also provided by three collaborating laboratories: 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 new mass spectrometry technique that provides results independent of a chromatographic separation.[12] FDA results were based on LC/MS/MS, and ChromaDex results were based on LC/UV absorbance.[7] Collaborating laboratories analyzed three or six subsamples of each SRM.

NIST Analyses for the Determination of Ephedrine Alkaloids. A variety of extraction approaches and conditions have been reported for the ephedrine alkaloids from herbal and dietary supplement samples. Jian et al. observed that different extraction techniques provided similar results for plant samples processed

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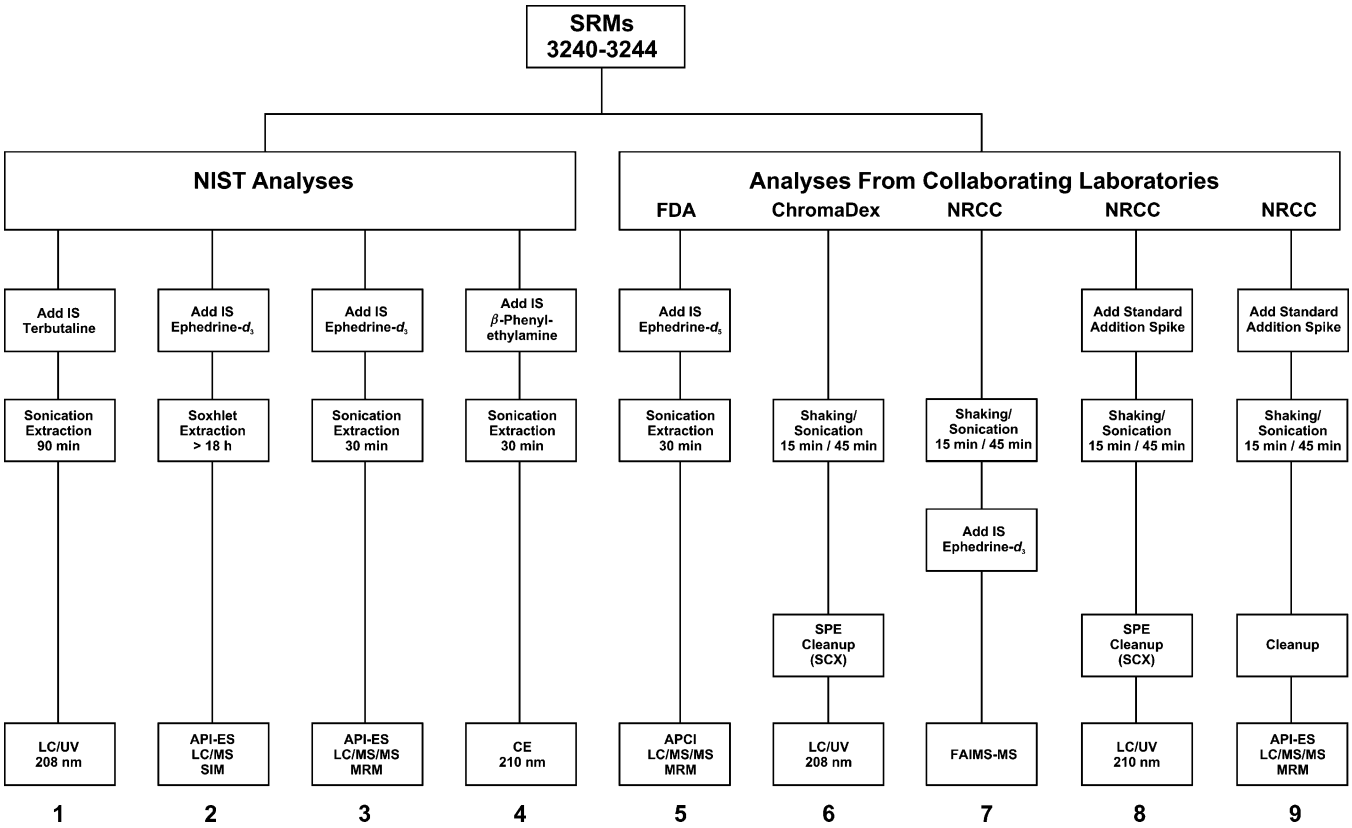

Figure 2. Analytical approaches used in the determination of ephedrine alkaloids in SRMs 3240−3244.

by 12-h cold maceration, 2-h reflux, or 40-min ultrasonic extractions.[13] McCooeye et al. described a pressurized fluid extraction procedure for the determination of ephedrine alkaloids in diet pills.[12] Comparable results were reported for extraction by shaking and sonication (15 min each). Hurlbut et al. extracted samples by stirring with a magnetic stirrer for 20 min,[14] and Sagara et al. used a 15-min reflux procedure at 85 °C.[15] Sagara and co-workers found that the levels extracted were constant after 15 min and that there was little difference between the use of methanol and water as extraction solvents. Acidic and basic extractions have also been reported. Basic conditions may facilitate the extraction of the free base form of the alkaloids (useful for GC analyses), whereas acidic conditions may increase analyte solubility in polar solvents and may help to release the analytes from the matrix. Two extraction approaches were used for NIST measurements: sonication extraction for 30 or 90 min and Soxhlet extraction for at least 18 h. Methanol was used in both methods. These conditions were selected to provide quantitative extraction of the ephedrine alkaloids and to provide method independence.

A number of reversed-phase LC methods for the determination of ephedrine alkaloids have appeared in the literature over the past 20 years. Many of these methods are based on ion pair chromatography with lauryl sulfate or sodium dodecyl sulfate in conjunction with C_{18} or CN columns. The concentration and source of the ion pairing reagent, mobile-phase pH, and column selection were found to significantly influence these separations. Other methods have been described based on high ionic strength, low-pH mobile phases, which are required to mask residual silanol interactions with the alkaloid amines. Most of the methods have been developed with phenyl stationary phases. Gay et al.[8] studied how phenyl stationary-phase loading affects selectivity toward the ephedrine alkaloids. By comparison, separations on C_{18} columns offer reduced peak symmetry and poorer resolution of methylephedrine and methylpseudoephedrine. Gay et al.[8] modified the method of Hurlbut et al.[14] to eliminate triethylamine from the mobile phase to improve ionization efficiency for LC/MS. Roman substituted a phosphate salt for the acetate buffer to improve detection at 210 nm.[7] In the current work, temperature was optimized for the method of Roman[7] to maximize resolution of norephedrine, norpseudoephedrine, and pseudoephedrine from matrix interferences.

The use of internal standards for LC/UV methods has been reported: phentermine,[4] amphetamine-*d*,[16] and butyl *p*-hydroxybenzoate.[17] In the NIST work, terbutaline was selected as an internal standard for LC/UV based on the retention characteristics and structural similarities to ephedrine. Terbutaline elutes slightly before ephedrine and is resolved from both the ephedrine alkaloids and matrix constituents of botanical and finished-product samples. A ~1 mg/g solution of terbutaline was used to spike the samples prior to extraction, at levels slightly less than those of ephedrine.

Due to the complexity of plant and finished-product matrixes, approaches have incorporated a SPE cleanup step to isolate the

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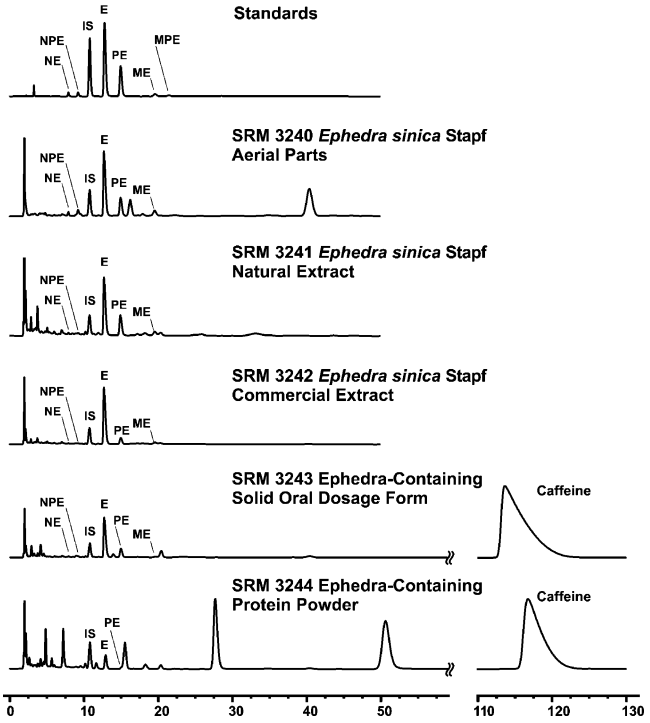

Figure 3. LC/UV analyses of the five ephedra-containing SRMs, with detection at 208 nm (NIST method 1). Component abbreviations are identified in Figure 1.

ephedrine alkaloids. Hurlbut[14] utilized a propylsulfonic acid SPE column to selectively retain the alkaloids prior to analysis. Roman[7] used a similar approach, but with different elution conditions. Both methods used an external standard approach to quantification. Gay and co-workers used the SPE method of Hurlbut with a stable isotope form of ephedrine as an internal standard for LC/MS, to compensate for processing losses. In initial method development efforts, we examined the use of SPE cleanup and found little improvement in the reduction of matrix interferences that affected the measurement of the ephedrine alkaloids; however, greater concern was placed on biases that could potentially be introduced by the use of a nonisotopically labeled internal standard (i.e., terbutaline). For these reasons, samples were not processed by SPE cleanup, and instead, extracts were filtered and directly injected.

Typical LC/UV separations of the five ephedra-containing SRMs using NIST method 1 are illustrated in Figure 3. Separations of SRM 3240 exhibited moderate matrix complexity. Slightly lower complexity is evident in the corresponding extract samples, SRM 3241 and SRM 3242. More significant interferences are present in the separations of SRM 3243 and SRM 3244, particularly for the minor alkaloid constituents. Both SRM 3243 and SRM 3244 contain significant levels of caffeine. Under isocratic elution conditions, caffeine elutes after ~2 h, and run times were extended correspondingly. Interferences from the matrix of SRM 3243 preclude determination of synephrine by this method.

Several major, unidentified constituents are present in the plant and protein powder SRMs. It is interesting that the component that elutes at ~40 min in SRM 3240 is also apparently present in SRM 3243 and SRM 3244 at low levels, even though there is no direct linkage between the two materials. This might be expected, however, if the dry herb were utilized to formulate the dietary supplements. A component in SRM 3240 elutes just after pseudoephedrine and is baseline resolved. This component is not observed in the other materials. Similarly, an unidentified component elutes just prior to pseudoephedrine in SRM 3243 and is not found in the other materials. Large responses are also observed at 28 and 51 min for SRM 3244. These components do not directly interfere with the determination of the ephedrine alkaloids for the LC/UV method, but extended run times are required to elute caffeine and other matrix constituents to prevent carryover of these components from run to run. The relative retention of these matrix constituents was observed to change with column temperature, and the best overall separations of analytes and matrix interferences were at 29 °C.

Typical LC/MS separations of the five ephedra-containing SRMs using NIST method 2 are shown in Figure 4. In general, the LC/MS method was free from interferences and provided a sensitive and robust approach to the determination of the ephedrine alkaloids. The mass fragments used in the LC/MS method were selected based on specificity and abundance of the ions. For synephrine (m/z = 150), norephedrine and norpseudoephedrine (m/z = 134 and/or 152), and ephedrine and pseudoephedrine (m/z = 148 or 166), $[M - 17]^+$ mass fragments were used, although strong $[M + H]^+$ signals were also observed. Better response for methylephedrine and methylpseudoephedrine was observed for $[M + H]^+$ ions (m/z = 180) than $[M - 17]^+$ ions. This choice was particularly important for the measurement of methylephedrine in SRM 3244, which is present at a low level. Even so, an interference with methylephedrine is present in SRM 3244 (see Figure 4), and manual integration was required. As expected, synephrine was detected only in SRM 3243 Ephedra-Containing Solid Oral Dosage Form. At least two of the dietary supplement finished products used in the preparation of this SRM were labeled to contain *Citrus aurantium*, a botanical source of synephrine.

LC/MS/MS separations (NIST method 3) are illustrated in Figure 5. These separations are superficially similar to the LC/MS SIM chromatograms; however, the separations represent MRM of $[M + H]^+$ ions, i.e., ions collisionally dissociated and fragmented to produce secondary ions characteristic of the precursor ions. For each of the analytes, the transition $[M + H]^+ \rightarrow [M - 17]^+$ was selectively monitored. Better sensitivity was obtained with this method, and measurement of methylephedrine and methylpseudoephedrine was possible in SRM 3244.

Chiral separations of the five ephedra-containing SRMs were carried out by CE, with three different chiral selectors (Figure 6). Only ephedrine and pseudoephedrine were targeted in these measurements due to limitations in detection sensitivity. The details of this method are reported separately;[9] however, three different chiral selectors were used to provide different chiral and achiral selectivity to resolve potential matrix interferences from the analytes. The results of these analyses show that only the naturally occurring enantiomers (−)-ephedrine and (+)-pseudoephedrine are present in the ephedra-containing SRM suite. Consistent results were obtained for the three CE methods, and measurements of the chiral alkaloids were combined with achiral measurements during value assignment.

Homogeneity was assessed from measurements carried out by LC/UV (method 1) and LC/MS (method 2). Six samples were

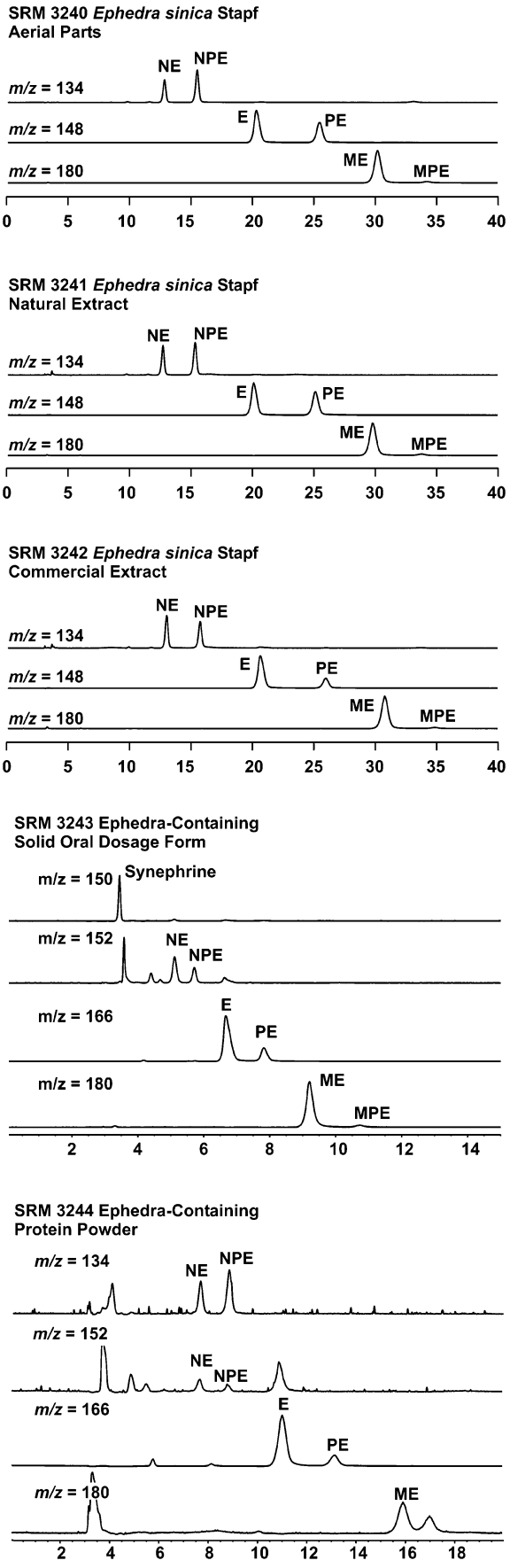

Figure 4. Positive ion, API-ES LC/MS analyses of SRMs 3240−3244 (NIST method 2). Selected-ion chromatograms are shown for *m*/*z* = 150, 134 or 152, 148 or 166, and 180. Component abbreviations are identified in Figure 1.

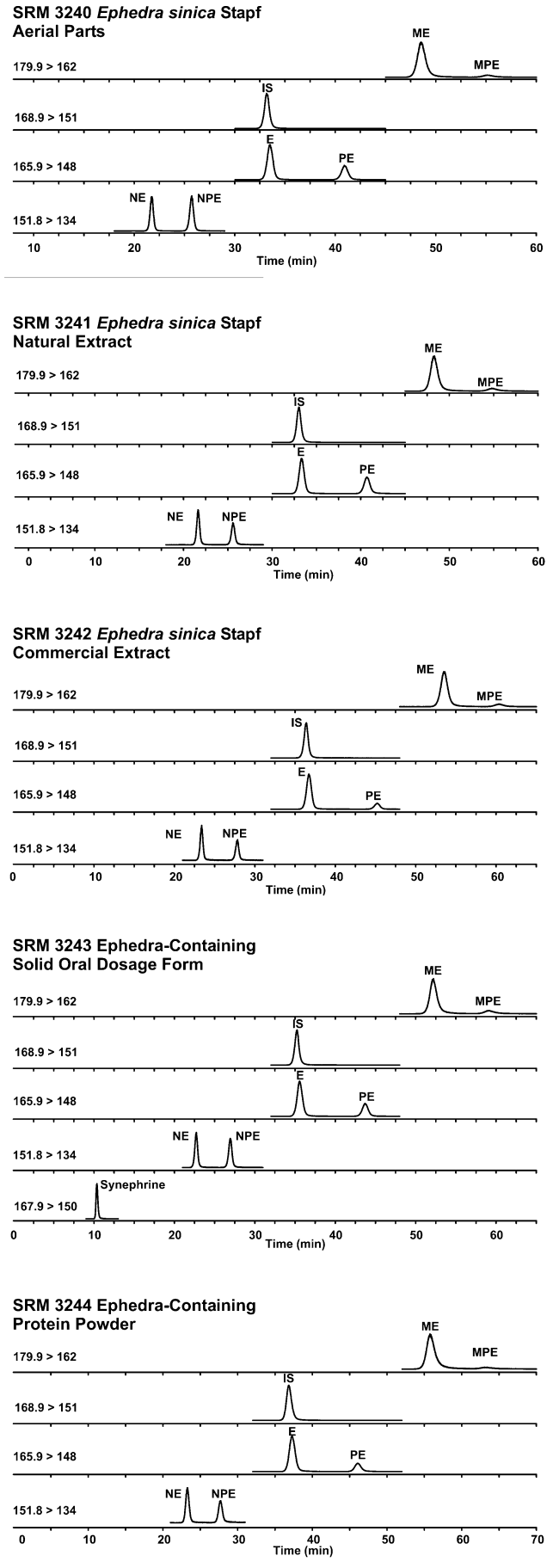

Figure 5. LC/MS/MS analyses of SRMs 3240−3244 (NIST method 3). Abbreviations are as in Figure 1.

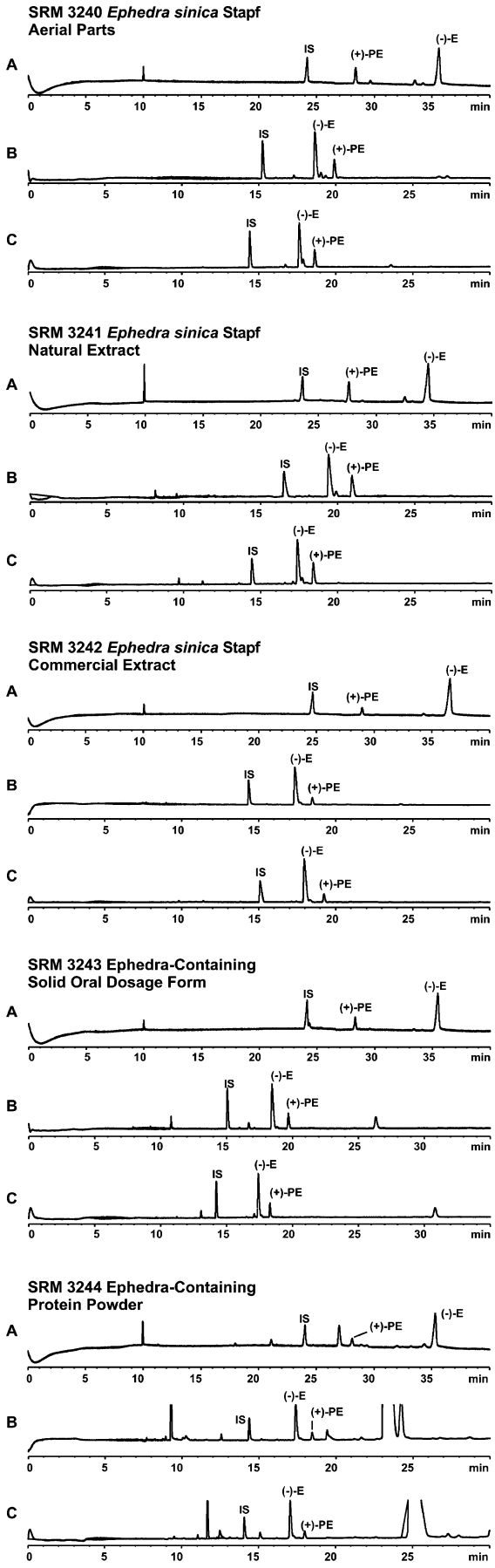

Figure 6. Chiral CE analyses of SRMs 3240−3244, for three different chiral selectors (NIST method 4). Detection is at 210 nm. Abbreviations are as in Figure 1.

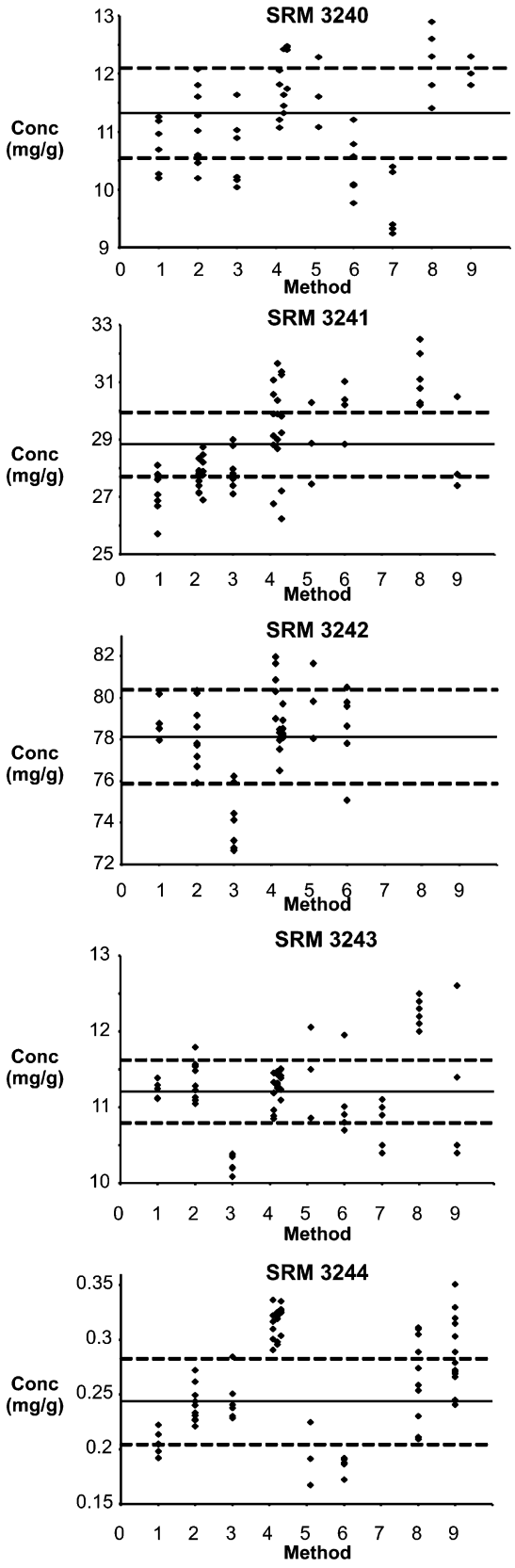

Figure 7. Individual measurements of ephedrine in SRMs 3240−3244, as determined by nine different methods. Certified values are indicated by solid lines; dashed lines represent the expanded uncertainty of the certified values. Method 1, NIST LC/UV; method 2, NIST LC/MS; method 3, NIST LC/MS/MS; method 4, NIST CE (chiral); method 5, FDA LC/MS/MS; method 6, ChromaDex LC/UV; method 7, NRC Canada FAIMS; method 8, NRC Canada LC/UV; method 9, NRC Canada LC/MS/MS.

Table 1. Averages and Standard Deviations of Measurements of Ephedrine Alkaloids (mg/g) in SRMs 3240−3244, As Determined by Different Analytical Approaches[a]

	NIST LC/UV			NIST LC/MS			NIST LC/MS/MS			NIST CE			FDA LC/MS/MS		
method	av	*s*	*N*	av	*s*	*N*	av	*s*	*N*	av	*s*	*N*	av	*s*	*N*
SRM 3240															
norephedrine	0.32	0.01	6	0.26	0.04	10	0.46	0.07	6				0.43	0.04	3
norpseudoephedrine	0.46	0.05	6	0.34	0.07	10	0.67	0.10	6				0.67	0.08	3
ephedrine	10.76	0.46	6	11.14	0.67	10	10.66	0.63	6	12.39	0.93	18	11.66	0.61	3
pseudoephedrine	3.30	0.18	6	3.18	0.30	10	3.63	0.30	6	3.83	0.35	18	3.29	0.20	3
methylephedrine	1.33	0.10	6	0.87	0.08	10	1.28	0.16	6				1.21	0.08	3
methylpseudoephedrine				0.043	0.004	10	0.06	0.01	6						
total alkaloids	16.26	0.79	6	15.89	1.09	10	16.84	1.05	6				17.26	0.73	3
SRM 3241															
norephedrine	0.26	0.01	7	0.31	0.04	17	0.45	0.02	7				0.50	0.01	3
norpseudoephedrine	0.28	0.02	7	0.28	0.05	17	0.45	0.03	7				0.55	0.04	3
ephedrine	27.13	0.81	7	27.83	0.51	17	27.96	0.70	7	29.43	1.56	18	28.87	1.42	3
pseudoephedrine	10.61	0.32	7	8.61	0.31	17	11.00	0.32	7	11.51	0.52	18	9.88	0.72	3
methylephedrine	2.42	0.13	7	1.72	0.21	17	2.80	0.13	7				3.04	0.17	3
methylpseudoephedrine				0.09	0.02	17	0.15	0.01	7						
total alkaloids	40.87	1.24	7	38.98	0.66	17	43.01	1.06	7				42.83	1.33	3
SRM 3242															
norephedrine	0.42	0.02	5	0.47	0.04	10	0.52	0.05	7				0.64	0.03	3
norpseudoephedrine	0.23	0.02	5	0.33	0.05	10	0.46	0.05	7				0.56	0.06	3
ephedrine	78.69	0.91	5	78.15	1.45	10	74.20	1.45	7	79.24	1.74	18.00	79.84	1.80	3
pseudoephedrine	9.29	0.41	5	9.24	0.60	9	10.60	0.70	7	9.57	0.41	18.00	7.83	0.83	3
methylephedrine	2.41	0.02	5	2.16	0.13	9	3.20	0.25	7				3.01	0.24	3
methylpseudoephedrine	0.01	0.01	4	0.09	0.01	10	0.16	0.02	7						
total alkaloids	91.59	1.31	5	90.72	1.66	9	89.66	1.98	7				91.90	2.18	3
SRM 3243															
synephrine				0.37	0.04	10	0.60	0.06	6				0.58	0.03	3
norephedrine	0.129	0.003	6	0.20	0.01	10	0.14	0.01	6				0.15	0.00	3
norpseudoephedrine	0.119	0.002	6	0.20	0.02	10	0.19	0.01	6				0.20	0.01	3
ephedrine	11.21	0.12	6	11.37	0.25	10	10.26	0.12	6	11.27	0.21	18	11.47	0.60	3
pseudoephedrine	2.67	0.06	6	2.75	0.11	10	3.06	0.10	6	2.82	0.11	18	2.72	0.21	3
methylephedrine	0.29	0.01	6	0.38	0.02	10	0.35	0.02	6				0.34	0.04	3
methylpseudoephedrine	0.061	0.003	6	0.026	0.002	10	0.024	0.003	6						
total alkaloids	14.42	0.12	6	14.87	0.37	10	14.10	0.24	6				14.88	0.60	3
SRM 3244															
norephedrine	0.0019	0.0002	6	0.0010	0.0002	10	0.0046	0.0004	6						
norpseudoephedrine				0.0013	0.0003	10	0.0047	0.0005	6						
ephedrine	0.21	0.01	6	0.24	0.02	10	0.25	0.02	5	0.32	0.01	18	0.19	0.03	3
pseudoephedrine	0.0296	0.0023	6	0.0317	0.0031	10	0.0517	0.0045	6	0.0499	0.0019	16	0.0246	0.0045	3
methylephedrine	0.0089	0.0029	6	0.0053	0.0006	10	0.0099	0.0009	6						
methylpseudoephedrine							0.00037	0.00004	6						
total alkaloids	0.25	0.01	6	0.28	0.02	10	0.32	0.03	6						

	ChromaDex LC/UV			NRCC FAIMS			NRCC LC/UV			NRCC LC/MS/MS		
method	av	*s*	*N*	av	*s*	*N*	av	*s*	*N*	av	*s*	*N*
SRM 3240												
norephedrine	0.50	0.02	6	0.46	0.07	6	0.53	0.04	6	0.57	0.10	6
norpseudoephedrine	0.68	0.08	6	0.72	0.11	6	0.76	0.09	6	0.89	0.12	6
ephedrine	10.42	0.54	6	9.85	0.57	6	12.13	0.56	6	12.78	0.87	6
pseudoephedrine	3.27	0.20	6	3.30	0.20	6	3.82	0.24	6	4.18	0.18	6
methylephedrine	1.04	0.04	6	1.34	0.11	6	1.28	0.06	6	1.12	0.08	6
methylpseudoephedrine							0.041	0.002	6	0.04	0.01	6
total alkaloids	15.90	0.85	6	15.66	0.91	6	18.56	0.98	6	19.58	1.08	6
SRM 3241												
norephedrine	0.77	0.06	6				0.63	0.03	6	0.64	0.05	3
norpseudoephedrine	0.42	0.03	6				0.70	0.06	6	0.61	0.12	3
ephedrine	29.92	0.89	6				31.15	0.93	6	28.57	1.69	3
pseudoephedrine	11.41	0.32	6				12.13	0.46	6	11.80	1.15	3
methylephedrine	2.93	0.06	6				2.73	0.09	6	2.53	1.47	3
methylpseudoephedrine							0.09	0.01	6	0.11	0.01	3
total alkaloids	45.44	1.27	6				47.43	1.41	6	44.26	4.41	3
SRM 3242												
norephedrine	0.79	0.09	6									
norpseudoephedrine	0.41	0.03	6									
ephedrine	78.58	1.95	6									
pseudoephedrine	9.09	0.17	6									
methylephedrine	3.06	0.11	6									
methylpseudoephedrine												
total alkaloids	91.93	2.30	6									

Table 1 (Continued)

	ChromaDex LC/UV			NRCC FAIMS			NRCC LC/UV			NRCC LC/MS/MS		
method	av	*s*	*N*	av	*s*	*N*	av	*s*	*N*	av	*s*	*N*
SRM 3243												
synephrine	0.62	0.04	6									
norephedrine	0.22	0.01	6	0.14	0.02	6	0.16	0.06	6	0.15	0.01	4
norpseudoephedrine	0.15	0.01	6	0.22	0.03	6	0.198	0.004	6	0.21	0.01	4
ephedrine	11.03	0.47	6	10.83	0.31	6	12.25	0.19	6	11.23	1.02	4
pseudoephedrine	2.78	0.12	6	2.63	0.10	6	2.95	0.07	6	2.93	0.34	4
methylephedrine	0.28	0.01	6				0.32	0.01	6	0.31	0.01	4
methylpseudoephedrine							0.012	0.001	6	0.016	0.002	4
total alkaloids	14.45	0.42	6				15.88	0.21	6	14.84	1.40	4
SRM 3244												
norephedrine										0.0042	0.0005	14
norpseudoephedrine							0.0033	0.0005	10	0.0046	0.0004	14
ephedrine	0.19	0.01	6				0.27	0.04	10	0.29	0.03	14
pseudoephedrine	0.0259	0.0010	6				0.0360	0.0040	10	0.0398	0.0033	14
methylephedrine							0.0072	0.0010	3	0.0062	0.0007	14
methylpseudoephedrine										0.00019	0.00003	7
total alkaloids										0.34	0.04	14

[a] av, average. *s*, standard deviation.

Table 2. Certified, Reference, and Information Values for Ephedrine Alkaloids in SRMs 3240–3244 (mg/g)[a]

	SRM 3240		SRM 3241		SRM 3242		SRM 3243		SRM 3244	
synephrine							0.54 ± 0.19	(35)		
norephedrine	0.44[b] ± 0.09	(20)	0.48[b] ± 0.20	(42)	0.57[b] ± 0.18	(32)	0.160[b] ± 0.026	(16)	0.0030[c]	
norpseudoephedrine	0.65[b] ± 0.14	(22)	0.44[b] ± 0.17	(38)	0.40[b] ± 0.16	(40)	0.186[b] ± 0.029	(16)	0.0034[c]	
ephedrine	11.31 ± 0.76	(6.8)	28.86 ± 1.17	(4.1)	78.1 ± 2.3	(2.9)	11.21 ± 0.42	(3.8)	0.242 ± 0.038	(16)
pseudoephedrine	3.53 ± 0.26	(7.5)	10.74 ± 1.11	(10)	9.27 ± 0.94	(10)	2.81 ± 0.11	(4.0)	0.036 ± 0.009	(24)
methylephedrine	1.18 ± 0.14	(12)	2.61 ± 0.51	(20)	2.77 ± 0.57	(20)	0.323 ± 0.031	(9.7)	0.0075[b] ± 0.0024	(31)
methylpseudoephedrine	0.046[b] ± 0.015	(33)	0.11[b] ± 0.09	(83)	0.124 ± 0.044	(35)	0.020[b] ± 0.011	(54)	0.0003[c]	
total alkaloids	17.0 ± 1.2	(6.0)	43.3 ± 2.7	(6.1)	91.2 ± 2.0	(2.2)	14.78 ± 0.54	(3.7)	0.296 ± 0.067	(23)

[a] Values in parentheses represent $(U/\bar{x}, \%)$. Each certified concentration value, expressed as a mass fraction on a dry-mass basis, is an equally weighted mean of the results from two to nine analytical methods carried out at NIST and at collaborating laboratories. The uncertainty in the certified value is expressed as an expanded uncertainty (U) about the mean ($\bar{x}$), following the ISO *Guide to the Expression of Uncertainty in Measurement*.[21] 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 ~95% confidence for each analyte. [b] Reference Values. [c] Information values, uncertainties are not provided due to limited data sets.

analyzed by method 1 and 10 samples were analyzed by method 2. Bottles for all analyses were selected on a fill basis using a stratified random sampling scheme. No indications of bottle-to-bottle inhomogeneity of the alkaloids were apparent in any of the data sets, for all SRMs, and no trends were apparent in graphs of analyte levels plotted as a function of bottle fill order.

Analyses by Collaborating Laboratories. During the development of the ephedra-containing SRM suite, Collaborative Studies were undertaken by AOAC International (Gaithersburg, MD) to validate two methods for the measurement of ephedrine alkaloids in complex sample matrixes. One study endeavored to validate the LC/UV method of Roman,[18] and another study undertook the validation of a LC/MS/MS method reported Sullivan et al.[19] These collaborative studies are ongoing; however, the methods under study are closely related to methods utilized by NRCC, FDA, and NIST (i.e., LC/MS/MS) and ChromaDex (LC/UV) to measure ephedrine alkaloids in SRMs 3240–3244.

The LC/MS/MS method used by FDA is a modification of the LC/MS method published by Gay et al. to utilize tandem mass spectrometry.[8,19] Three MRM transitions were monitored based on the $[M + H]^+$ precursor ion to prove the identity of each component. Quantification was based on the sum of ion currents from three characteristic secondary ions: $[M + H - 18]^+$; $[M + H - 33]^+$; $[M + H - 49]^+$. In general, method precision was excellent. Coefficients of variation (CVs) ranged from ~2% to 10% for ephedrine and pseudoephedrine, to ~1% to 12% for norephedrine, norpseudoephedrine, and methylephedrine. Somewhat poorer precision was obtained for SRM 3244 Ephedra-Containing Protein Powder. Levels of the alkaloids are significantly lower in this material than in the other SRMs, and the protein matrix is more complex than in the other SRMs. This is a general trend observed for all of the methods and laboratories that is expected: measurement precision is better for more concentrated analytes and simpler sample matrixes.

The LC/MS/MS method of NRCC is comparable to NIST's method 3, with differences in sample extraction and cleanup. The biggest difference is in the approach to quantification: the NIST method used a stable isotope internal standard, and NRCC used

(18) Castor, T. P. AOAC International Collaborative Study Protocol for HPLC–UV Determination of Ephedra Alkaloids in Botanicals and Dietary Supplements. Ka012, 1–86. AOAC Int.: Gaithersburg, MD, 2002.

(19) Sullivan, D.; Wehrmann, J.; Schmitz, J.; Crowley, R.; Eberhard, J. *J. AOAC Int.* **2003**, *86*, 471–5.

a standard addition method without an internal standard. CVs for ephedrine and pseudoephedrine ranged from 4% to 12%, and CVs for the minor alkaloids ranged from 4% to 20%. Better precision was obtained for the more concentrated samples.

NRCCs LC/UV method (method 8) is comparable to ChromaDex's LC/UV method (method 6), with the main differences being the approach to extraction and quantification. As with their LC/MS/MS method, NRCC used standard additions for quantification, in contrast to ChromaDex's use of a single-point calibration. Neither method incorporated an internal standard. The NRCC method CVs for ephedrine and pseudoephedrine were typically 2%−7% (13% for SRM 3044), whereas CVs for the minor alkaloids ranged from 5% to 23%, depending on component concentration and sample matrix. The LC/UV method of ChromaDex produced consistently precise results: CVs ranged from 2% to 6% for most analytes; however, levels of the minor alkaloids were not reported for SRM 3244.

The FAIMS−MS method of NRCC is unique in that it does not depend on a chromatographic separation to resolve the three pairs of diastereomeric alkaloids. This method utilized a stable isotope internal standard for quantification. The precision (CVs) of ephedrine and pseudoephedrine measurements was excellent: 3%−6%. As observed with the other methods, the precision of the minor alkaloids was somewhat poorer: 8%−15%. Methylpseudoephedrine was not determined by this approach.

Comparison of Results and Assignment of Certified Values. Results of the analyses of the five SRMs for ephedrine alkaloids are summarized in Table 1 for the nine methods, and certified values for the alkaloids are provided in Table 2. In most cases, the certified values in the SRMs are the equally weighted means of the mean results from the nine methods as available (not all samples or analytes were determined with each method). For SRM 3242 and SRM 3244, values for methylpseudoephedrine were determined by a "bound on bias" methodology (BOB), which may be more appropriate for smaller data sets.[20] The associated uncertainties are expanded uncertainties at the 95% level of confidence with a coverage factor determined from the Student's *t* distribution corresponding to the appropriate associated degrees of freedom, calculated according to the ISO Guide.[21] Agreement among methods was very good overall. Method uncertainties (CV) typically ranged from ∼2% for high-level analytes to ∼15% for trace-level measurements. Method bias was not observed for data used in value assignment. Plots of individual measurements for ephedrine are provided in Figure 7. This figure is representative of the scatter observed within and among methods for the higher level alkaloids. Similar plots of results for the other alkaloids, and results for total alkaloid content, are provided as Supporting Information (Figures S1−S6). As expected, better agreement was obtained for measurements made for more concentrated constituents (i.e., ephedrine, pseudoephedrine, and total alkaloids) compared with the minor constituents (norephedrine, norpseudoephedrine, methylephedrine, and methylpseudoephedrine). The poorest agreement was obtained for SRM 3244 Ephedra-Containing Protein Powder, for which levels of the ephedrine alkaloids were 20−400 times lower than in the other materials, and interferences from the sample matrix were significant. Even so, three of the six alkaloids have been certified in this material based on the combined data.

CONCLUSIONS

SRMs 3240−3244 represent the first in a series of dietary supplement SRMs to be offered by NIST with certified values for organic constituents and selected trace elements. These materials are provided primarily for use in method development and as control materials to support analytical methods for the determination of these constituents. In the absence of a formal regulatory environment, the SRM suites will assist manufacturers of dietary supplements to characterize raw materials voluntarily, to prevent the use of materials that are contaminated or adulterated. In addition, the SRMs will assist self-assessment of consistency and quality in finished products. The goal of this ongoing effort is to provide tools to the dietary supplement industry and measurement communities that will lead to improved quality of dietary supplements and ultimately reduce public health risks that could potentially be associated with these products.

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SUPPORTING INFORMATION AVAILABLE

Additional figures showing individual measurements of specific analytes in SRMs 3240−3244, as determined by up to nine different methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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