The Perils of Paula, Episode 2

NIST specialist reviews certified reference materials.

By Paula Brown & Dr. Katherine Sharpless

Paula Brown, Program Head of the Natural Health Product Research Group (NRG) at BCIT, directs a multi-disciplinary research facility providing fundamental investigations on product quality, safety and efficacy. In establishing NRG, Ms. Brown has created the capacity to support industry through the entire continuum, from grower to manufacturer, ensuring product integrity is maintained. An active volunteer, she is a founding member of the NHP Research Society of Canada, a non-profit group dedicated to supporting and promoting scientifically rigorous research and education on NHPs. Ms. Brown is also General Referee for the AOAC Dietary Supplements Methods Committee. She can be reached at BCIT, 3700 Willingdon Avenue, Burnaby, BC, Canada, V5G 3H2, E-mail: pbrown@bcit.ca; Website: www.bcit.ca/appliedresearch.

Katherine Sharpless is an analytical chemist and the scientific assistant of the Analytical Chemistry Division, Chemical Science and Technology Laboratory, National Institute of Standards and Technology (NIST), Gaithersburg, MD. She can be reached at katherine.sharpless@nist.gov.

Previously on…

During the last "Quality Focus" column (April 2010) Dr. Joseph Betz and I tried to set the stage for reference materials within the context of GMPs, provide some basic definitions and introduce the chemical calibration standard, one type of Reference Material. While it was suggested you should "tune in" this month to read about botanical reference materials, from the title you have probably guessed that I will not be tackling that topic. This is mainly for two reasons: (1) I didn't do justice to a key reference material cast member, the ***Certified Reference Material*** and (2) the botanist escaped to a tropical Island off the South East coast of Africa. Luckily, we have special guest and reference material guru Dr. Katherine Sharpless to walk us through NIST's certified reference material program and provide updates on its progress with dietary supplement materials.

Enter Stage Left: Certified Reference Materials

This month, the final stage of the staggered implementation of current Good Manufacturing Practices (cGMPs) took effect, requiring all domestic and foreign companies that manufacture, package, label or hold dietary supplements to comply with its rules. The National Institute of Standards and Technology (NIST) is working with the National Institutes of Health's Office of Dietary Supplements (NIH-ODS) and FDA to underpin the quality of dietary supplement analyses—in both raw ingredients and finished products. For those who are unfamiliar with NIST, it is a non-regulatory agency of the U.S. Department of Commerce. NIST's mission is to promote U.S. innovation and industrial competitiveness by advancing measurement science, standards and technology in ways that enhance economic security and improve quality of life. In other words, we're from the government, and we're here to help you. One of the ways we do this is through the production of Standard Reference Materials.

A reference material is a homogeneous material characterized for use as a calibrant, for assessment of an analytical method, or for assigning values to other materials. These materials are also often called "standards." A certified reference material (CRM) is a reference material that's accompanied by a certificate that provides certified values and uncertainties for a particular property of the material, and establishes traceability to an accurate realization of the unit in which the property values are expressed [VIM]. In the field of dietary supplements, CRMs are most likely to take one of three forms: a substance of certified purity (for use in preparation of calibration solutions, for example), a calibration solution itself or a matrix material that has been characterized for active or marker compounds. NIST's CRMs are known as Standard Reference Materials (SRMs).

NIST has produced a variety of natural-matrix SRMs intended for use by the dietary supplement community (*see Table 1*), and production of additional materials, and now also calibration solutions, continues. Many of the natural-matrix materials are provided as suites that represent different analytical challenges: (1) plant material that is often difficult to extract because of incorporation of analytes inside cell walls; (2) an extract, which might be the form used by a finished-product manufacturer and which often easily dissolves in water or another sol-

vent; and (3) a finished product, which likely incorporates an extract of the plant of interest but the analysis of which may be challenging because of the presence of other ingredients. These SRMs are characterized for active or marker compounds and, in some cases, toxic elements (As, Cd, Hg and Pb). These materials have also been screened for pesticides, but

Table 1. *A list of materials that are currently available or that are expected to be available within the next three months.*

SRM Number	Name	Analytes for Which Values are Assigned
3240	*Ephedra sinica* Stapf Aerial Parts	Ephedrine alkaloids, toxic elements
3241	*Ephedra sinica* Stapf Native Extract	Ephedrine alkaloids, toxic elements
3242	*Ephedra sinica* Stapf Commercial Extract	Ephedrine alkaloids, toxic elements
3243	Ephedra-Containing Solid Oral Dosage Form	Ephedrine alkaloids, caffeine, toxic elements
3244	Ephedra-Containing Protein Powder	Ephedrine alkaloids, caffeine, toxic elements, nutrients
3245	Ephedra Dietary Supplement Suite	(Two bottles of each of the ephedra materials listed above see analytes listed above)
3246	*Ginkgo biloba* (Leaves)	Flavonoids, terpene lactones (ginkgolides), toxic elements
3247	*Ginkgo biloba* Extract	Flavonoids, terpene lactones (ginkgolides), toxic elements
3248	Ginkgo-Containing Tablets	Flavonoids, terpene lactones (ginkgolides), toxic elements
3249	Ginkgo Dietary Supplement Suite	(Two bottles of each of the ginkgo materials listed above see analytes listed above)
3250	*Serenoa repens* (Fruit)	Fatty acids, phytosterols
3251	*Serenoa repens* Extract	Fatty acids, phytosterols, carotenoids, tocopherols
3254	*Camellia sinensis* (Green Tea Leaves)	Catechins, caffeine, theanine
3255	*Camellia sinensis* (Green Tea) Extract	Catechins, caffeine, theanine
3256	Green Tea-Containing Solid Oral Dosage Form	Catechins, caffeine, theanine
3258	Bitter Orange (Fruit)	Adrenergic amines
3259	Bitter Orange Extract	Adrenergic amines
3260	Bitter Orange-Containing Solid Oral Dosage Form	Adrenergic amines, caffeine
3261	Bitter Orange Dietary Supplement Suite	(Two packets of each of the bitter orange materials listed above see analytes listed above.)
3274	Botanical Oils Containing Omega-3 and Omega-6 Fatty Acids (Flax, Borage, Evening Primrose, Perilla)	Fatty acids
3275	Fish Oils Containing Omega-3 and Omega-6 Fatty Acids	Fatty acids
3278	Tocopherols in Edible Oils	Tocopherols, tocotrienols
3280	Multivitamin/Multielement Tablets	Vitamins and carotenoids (15), elements (18)
3281	*Vaccinium macrocarpon* (Cranberries)	Organic acids, sugars, nutrient elements. (Values for anthocyanidins and procyanidins will be added at a later date.)
3282	Cranberry Juice Cocktail	Organic acids, sugars, nutrient elements. (Values for anthocyanidins and procyanidins will be added at a later date.)
3283	*Vaccinium macrocarpon* (Cranberry) Extract	Organic acids. (Values for anthocyanidins and procyanidins will be added at a later date.)
3284	Cranberry-Containing Solid Oral Dosage Form	Organic acids. (Values for anthocyanidins and procyanidins will be added at a later date.)
3285	Mixed Berry-Containing Solid Oral Dosage Form	Organic acids. (Values for anthocyanidins and procyanidins will be added at a later date.)
3287	*Vaccinium corymbosum* (Blueberries)	Organic acids, nutrients. (Values for anthocyanidins and procyanidins will be added at a later date.)
3291	*Vaccinium myrtillus* (Bilberry) Extract	Organic acids. (Values for anthocyanidins and procyanidins will be added at a later date.)

Source: NIST, (For ordering and pricing information, visit http://www.nist.gov/srm.)

quantifiable levels have not been found in any of the materials thus far. A Certificate of Analysis (C of A) is generated, containing information about the material's intended purpose, how it was prepared and characterized and its assigned values, among other things. C of As may also contain additional information such as a chromatographic "fingerprint," microscopy or thin layer chromatograms. These SRMs are meant to be used as quality control materials for chemical testing or for development or validation of analytical methods, such as is required for GMPs. Although voucher specimens exist for most of the plant and extract SRMs, they are not meant to be used for identity testing, nor are they meant to represent what a "good" dietary supplement product should contain.

Subpart E of the GMPs says that for each ingredient in your product you must establish specifications for identity, purity, strength and composition, and set limits on contaminants and adulterants. Subpart J says you must follow processes for selection and use of appropriate analytical methods and reference materials. You must verify that the methods are appropriate for your intended use, then you must use those methods to determine whether you are meeting your product specifications. And this is where natural-matrix SRMs come in to play:

- You can analyze an SRM during method development. If your result agrees with the assigned value, then your method will probably give good values for other similar samples.
- You can analyze an SRM during method validation. You can analyze the SRM and other samples typical of those that you will analyze (matrix, concentration) to demonstrate your method's scope of applicability and that your method has acceptable accuracy, repeatability, and selectivity. (AOAC recommends analysis of at least three materials, at least in duplicate, on at least two days.)
- You can analyze an SRM for quality control (QC). You can prepare and analyze the SRM the same way you would an unknown sample. If your matrices are similar enough and the result for the SRM agrees with the assigned value, then your results for your own sample are probably right.
- And, finally, you can analyze an SRM to establish traceability. You can prepare and analyze the SRM along with an in-house QC material over a set timeframe to establish values for your in-house QC, and then use that material for routine quality assurance. Traceability is "…the property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties." (*ISO VIM, 1993*) "Traceable to NIST" is shorthand for saying that you have "Results of measurements that are traceable to reference standards developed and maintained by NIST."

When selecting an SRM (or any CRM) for these purposes, there are several factors to consider: the similarity of the matrix to your usual samples, which will influence your requirements for sample preparation and the likelihood of similar analyte recovery; the similarity of analyte levels to those in your usual samples; and whether or not values are assigned for the analytes you're interested in measuring. You should not limit yourself to considering just the materials shown in *Table 1.* If you're setting maximum levels for toxic elements in your products and your usual samples are leaves, you might consider the ephedra aerial parts or ginkgo leaves. But you should also look at analyte levels in SRM 1515 Apple Leaves or SRM 1570a Trace Elements in Spinach Leaves or another leaf SRM or other organizations' leaf CRMs to see whether one of these non-dietary supplement materials is a good match for your expected analyte level. If you're measuring pesticides or polycyclic aromatic hydrocarbons, you might consider a classically "environmental" CRM. And for vitamins and other nutrients, you might consider a food CRM.

Certainly for measurements of many active or marker compounds, you're going to need a sample representing the same species. If you're measuring ginkgolides in *ginkgo* leaves, for example, you are going to want a ginkgo leaf CRM. While the availability of CRMs for this type of situation is more limited, the list is growing.

Next time on…

Tune in to September's "Quality Focus" column to find out whether that mysterious "Botanist" character returned from the tropical island in time to help Paula meet her deadline! NW

References furnished upon request.