

TECHNICAL COMMUNICATIONS

Development of the Analytical Methods and Reference Materials Program for Dietary Supplements at The National Institutes of Health

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The need for validated analytical methods and reference materials to identify and measure constituents in dietary supplements is essential. Such methods allow for the documentation that products meet manufacturer's specifications and contain what their labels declare. In March 2003, the U.S. Food and Drug Administration issued a proposed rule that would establish specific current good manufacturing practices for dietary supplements. This proposed rule requires that companies create a quality control unit to set specifications and ensure compliance with these specifications using scientifically valid procedures. This report provides insights and lessons learned from 3 meetings the Office of Dietary Supplements (ODS) at the National Institutes of Health helped organize. These meetings were structured to (1) educate individuals about the importance and need for validated analytical methods and reference materials to identify and quantify constituents of dietary supplements; (2) identify resources required to fulfill this need; and (3) serve as a platform to obtain input from interested parties to help frame the research agenda for the Dietary Supplements Methods and Reference Materials Program within ODS. Stakeholder's opinions and views expressed at these 3 meetings are outlined in this report.

In 1994, Congress amended the Federal Food, Drug, and Cosmetic Act by passing the Dietary Supplement Health and Education Act (DSHEA; 1). This Act created a special category of foods called dietary supplements. Section 402(g)(2) of this Act granted the Secretary of the Department of Health and Human Services the authority to issue current good manufacturing practices (cGMPs) for dietary supplements. In issuing this permission to promulgate cGMP regulations, Congress stipulated that they be "modeled after cGMP regulations for food and may not impose standards for which

there is no current and generally available analytical methodology." The Act thus recognized the need to have publicly available analytical methods in place before standards could be imposed.

On March 13, 2003, the U.S. Food and Drug Administration (FDA) issued a proposed rule that would establish specific cGMPs for dietary supplements (2). One important provision of the proposed rule calls for companies to establish raw material and finished product specifications and perform tests to determine whether these specifications are being met. The proposed rule requires that these specifications be designed in a manner that provides assurance that dietary supplements are manufactured to ensure their identity, purity, strength, and composition. Tied to this provision is the requirement that companies create a quality control unit to establish specifications and evaluate compliance using scientifically valid procedures. These proposed requirements further emphasize the need for validated analytical methods and reference materials, which are necessary for compliance and enforcement of the GMP regulations.

In addition to establishing dietary supplements as a class of legal goods and granting the FDA the authority to develop GMPs, DSHEA also created the Office of Dietary Supplements (ODS) within the Office of the Director at the National Institutes of Health (NIH). Section 485C(c)(3) of Title IV of The Public Health Services Act directs ODS to "serve as the principal advisor to the Secretary and to the Assistant Secretary for Health and provide advice to the Director of the NIH, the Director of the Center for Disease Control and Prevention, and the Commissioner of Food and Drugs on issues relating to dietary supplements including: scientific issues arising in connection with the labeling and composition of dietary supplements" (1). Section 485C(c)(1) the Act states that ODS, "conduct and coordinate scientific research within NIH relating to dietary supplements and the extent to which their use can limit or reduce the risk of diseases and conditions such as heart disease, cancer, birth defects, osteoporosis, cataracts, and prostatism." If the findings from clinical studies are to be credible, test materials used in these studies must be reliable and well characterized. These materials then must adhere to stringent requirements that can be met only if validated analytical methods and reference materials are available.

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Recognizing the importance of these concerns, the U.S. Senate provided language and a mandate in its budget deliberations for fiscal year 2002 that called “for ODS to allocate sufficient funds to speed up an ongoing collaborative effort to develop and disseminate validated analytical methods and reference materials for the most commonly used botanicals and other dietary supplements” (3). As a consequence, the Dietary Supplements Methods and Reference Materials Program was initiated within ODS in 2002. The mandate was reaffirmed for fiscal year 2003 (4). The primary goal of this program is to provide industry, researchers, and regulators with analytical methods and reference materials that will assist them in meeting label claims, developing quality standards, complying with regulations, enforcing regulations, and ensuring the quality of test materials used in research.

Development and Validation of Analytical Methods and Reference Materials for Dietary Supplements

February 8, 2002, Stakeholders meeting.—This public meeting included representatives from the supplement industry (manufacturers, suppliers, and trade associations), analytical laboratories, regulatory and other governmental entities, nongovernmental organizations, and consumer groups. The main purpose was to seek comments that would help the ODS develop an umbrella strategy for research, development, validation, and dissemination of analytical methods and reference materials for its newly formed Methods and Reference Materials Program. The meeting began with 2 plenary presentations from ODS. Paul Coates, director of the Office, provided an introduction to activities of the Office; Joseph Betz, director of the newly formed program, outlined the specific questions which the Office was seeking comments on to help formulate the program: (1) Who are the various interested parties? Whom do they represent and what are their needs? (2) Are there tensions among the needs of these various groups, i.e., industry, regulators, researchers, consumers? (3) What should the goals of the ODS program be? (4) How should selection of methods/reference materials be prioritized (i.e., are there current safety, labeling, or other marketplace issues that ODS should consider in priority setting)? (5) What contributions are interested parties willing to make to the methods/reference materials development process?

During the discussions that followed, there was recognition that validated methods and reference materials are needed for dietary supplements; however, there were a number of comments regarding the process necessary to achieve this outcome. These included taking into consideration the complexity of developing validated methods and reference materials for botanicals. Botanical raw materials are chemically complex and their composition is often poorly characterized (5). In addition, the identities of the active constituent(s) in botanical supplements are often not known (6). As a result, chemotaxonomic markers are sometimes measured as indicators of botanical identity or as tools in manufacturing to help ensure consistency of products (7). Chemotaxonomy is the science of classifying living objects based on their chemical or

biochemical profiles (8). Some needs highlighted at the meeting were (1) to identify and quantify chemotaxonomic markers as well as components with known biological activity; and (2) to develop qualitative methods, such as thin-layer chromatography to fingerprint complex mixtures such as botanicals, to identify products spiked with pure compounds to meet quantitative label claims.

Included in the range of comments was that the Office recognized that validated methods that meet an *Official Methods of Analysis*SM standard may not be necessary in all cases, as different levels of validation may be necessary, depending on the end-use for that method, e.g., routine analysis for quality control versus regulatory enforcement action. While engaged in the process of validating methods, ODS should appreciate the efforts by other groups involved in the development and validation of methods, and consider existing methods that measure compounds in nonsupplement matrixes. For example, methods for determining toxic substances such as pesticides and mycotoxins have been developed for conventional food (9), but would need to be revalidated for dietary supplements. Participants also asked that a uniform guidance for validating methods be published as there are currently several recommendations from groups such as AOAC INTERNATIONAL, the U.S. Pharmacopeia (USP), and the International Committee on Harmonization.

Other comments were related to the development of reference materials, particularly for botanicals. For example, should these materials be original plant materials, i.e., voucher specimens that can be traced back to their source, or standardized extracts? Ongoing efforts by the University of Mississippi in acquiring reference materials and the National Institute of Standards and Technology (NIST) in developing Standard Reference Materials[®] (SRMs) were mentioned. The importance of scientifically valid methods for establishing quality standards was also raised, because poor testing methods can give rise to poorly conceived specifications for product quality. Finally, the need for standardized product testing to evaluate complementary and alternative therapies, to compare data from multiple studies, and to build the evidence base going from basic to clinical studies was raised.

Given the scope of work the ODS was asked to undertake, attendees stressed the need to have mechanisms in place to identify dietary supplement components for which methods are needed and a process to prioritize their development. The AOAC Dietary Supplement Task Group (DSTG; formed in 2000) was identified as a group that could oversee the development of validated methods, as it has wide representation of stakeholder groups and mechanisms to perform these tasks.

The following recommendations were repeatedly emphasized by stakeholders in the extended discussion session. They collectively suggested that the ODS pay more attention to basic quality issues such as determining components and contaminants in botanicals; accept the DSTG's recommended list of dietary supplements for which validated methods are needed; and evaluate approaches used for validating methods by organizations, such as AOAC and USP, before creating a new framework for validating methods.

Dietary Supplements Analytical Methods Workshop

April 18, 2002.—This workshop focused on raising the level of understanding of the importance of appropriately selecting, validating, and documenting analytical methods, depending on their end use, which is to appropriately meet the diverse needs of industry, basic and clinical researchers, and regulatory officials. As with the February 8, 2002, meeting, this workshop included wide representation from industrial, governmental, nongovernmental, and consumer groups. Representatives of the groups presented their perspectives on their internal mechanisms for selecting methods for validation and on reasons why validated methods and reference materials were important. These perspectives were presented in plenary sessions in the first part of the workshop. In the second part, attendees met in 3 breakout groups and were given the charge to discuss the morning sessions and to provide suggestions to ODS for implementing the Methods and Reference Materials Program.

In his keynote address, Bernard A. Schwetz, former acting principal deputy commissioner of the FDA, stressed the need for validated methods in clinical trials designed to evaluate the safety of dietary supplements. He reiterated that validated methods are required for the quality control of products, to lend credibility to the findings, for reproducibility of studies, to compare results across different trials, and to document the weight of scientific evidence about that product. He also noted that validated methods are most needed by FDA to help achieve its primary role in helping ensure the safety of the food supply.

The need for appropriately validated methods in research was reinforced by Marguerite Klein of the National Center for Complementary and Alternative Medicine (NCCAM). She discussed the challenges faced when designing clinical trials to validate complementary and alternative medicine treatments. Selecting a well-characterized, standardized product suitable for testing in these NIH sponsored clinical trials is vital to the success of the NCCAM mission, as NCCAM expects its grantees will use due diligence in selecting and evaluating test materials (10). A lack of validated analytical methods is a sizeable obstacle to such due diligence.

Karen Wolnik at FDA's Forensic Chemistry Center discussed challenges the Agency faces when surveying the U.S. marketplace for dietary supplements that pose safety concerns. The example provided was a survey of the marketplace for products that contain species of the genus *Aristolochia*, many of which contain aristolochic acid, a substance known to be nephrotoxic and carcinogenic. The FDA faced significant challenges in selecting the appropriate set of methods to analyze products that may contain one or more of the 14 aristolochic acid because of the limited availability of validated methods and difficulty in obtaining authenticated botanical samples. For the agency's purpose of ensuring safety, analytical methods must be extremely sensitive and selective, while avoiding both false-positive and -negative results.

Representatives from industry provided different perspectives in their response to the need for validated methods and reference materials. Among the issues manufacturers face is selecting a method for a raw material used to produce a dietary

supplement when the supplier of that raw material uses a different in-house method to certify the material supplied. In the absence of a single agreed-upon method, manufacturers may be placed in the awkward position of having to choose between 2 sets of analytical results for quality assurance and label claims. If regulatory officials choose a different method of analysis than that used by the manufacturer and that method yields different numerical results, manufacturers may be subject to regulatory action. One solution to this dilemma is to use an in-house method that has been validated against a reference method to verify that the same raw material from different suppliers meets internal specifications. This is a particularly challenging problem for an industry with limited laboratory resources. However, having a readily accessible source (e.g., Web-based) of validated analytical methods is very important to the industry, especially for independent laboratories that have to satisfy many customers and still remain independent and unbiased in providing third party certifications. It was noted by an industry spokesperson that the absence of minimum standards of identity, purity, and potency for supplement ingredients has contributed to the adulteration and misbranding of dietary ingredients, because some manufacturers do not set and meet specifications for their products.

In the breakout sessions, attendees reached the following conclusions and recommendations. They suggested that the nature and direction of the ODS program should focus on the following: (1) Identify a list of 20–25 dietary supplement components in need of validated analytical methods followed by timely development of these methods. While undertaking this effort, the tension between publicly available and proprietary methods must be recognized. (2) Use a consensus process consisting of a steering committee and small groups to help guide the identification and selection of methods. (3) Include mechanisms that reward innovative methods; currently there are few financial incentives that reward such innovation. (4) Develop qualitative methods (e.g., “fingerprinting”) for establishing and documenting plant identity. (5) Support basic research efforts to identify appropriate phytochemical markers and underlying mechanisms of action, as these are critical to the development of sound analytical methods. Such research would identify which of the compounds need to be measured.

Certified Reference Materials for Analysis of Botanical Dietary Supplements

September 22, 2002, TDRM symposium.—AOAC's Technical Division for Reference Materials (TDRM) symposium was a follow-up to the previous year's session entitled “Dietary Supplements: Analytical Methods and Reference Standards—What Next?” These TDRM symposia are held in conjunction with the AOAC Annual Meeting. Participants at the 2002 session stated that although the rate of development of certified reference materials (CRMs) had accelerated, the process was slow and did not keep up with the pace of development of validated analytical methods by AOAC and monographs by the USP and the American Herbal Pharmacopeia.

At the symposium, governmental officials from the United States and Canada described their efforts at developing reference materials and outlined the need for these reference materials by regulatory agencies. They stated that reference materials to accompany the corresponding analytical methods are vital to the standard-setting process and enforcement actions.

The symposium also focused on the mechanics for creating reference materials and an enumeration of activities that would enhance the availability of these materials. In addition to defining terms used to describe reference materials, presenters elaborated the various steps needed to convert large quantities of dried plant material into sealed vials of CRM. Stephen Wise (NIST, Gaithersburg, MD) described the types of reference materials offered for commercial use by NIST. His presentation focused on the SRM program, which issues SRMs accompanied by certificates of analysis. A major stumbling block to preparing these SRMs is a lack of reliable analytical methodology. NIST seeks guidance from the FDA when prioritizing the development of reference materials.

Agnes Nguyenpho of the FDA's Center for Drug Evaluation and Research provided insights into the difficulties of obtaining authentic botanical raw materials and raw material extracts from non-U.S. sources. She outlined the legal barriers and protections imposed by the U.S. Department of Agriculture and FDA, indicating the difficulties when these botanical ingredients are regulated as drugs or classified as narcotics or endangered species in the United States and the country of origin. Speakers from commercial reference material testing laboratories also provided perspectives on how a commercial laboratory creates chemical reference materials and the degree of characterization necessary for such materials to be useful to the analytical community.

Following the structured presentations, the panelists further highlighted some of the obstacles to the rapid development and dissemination of reference materials. They composed questions that should be asked when developing these materials, and some provided solutions to overcoming obstacles. Among the factors highlighted were, should these materials be single chemical entities or materials in a complex matrix? How comprehensive should the documents describing the source of these materials be? To what extent should one be able to trace these materials back to their origin? Is commercial production necessary or feasible? And, what comes first—validated methods or reference materials?

Conclusions

Results of these 3 meetings provide 4 important insights for those involved in selecting methods and reference materials for industrial, research, and compliance purposes: (1) When selecting a method, ensure that the method is designed and validated sufficiently for its end use, i.e., fit for purpose. (2) Scientifically valid methods are necessary for establishing quality standards and for measuring compliance with the standards, as poor quality testing methods can give rise to poorly conceived specifications for product quality.

Universally available validated methods are important to cross-verify that the same raw material from different suppliers meets the specifications set by the manufacturer. (3) When selecting a method for a raw materials or finished product, the identity of the active constituent(s) may be unknown; therefore, choice of appropriate chemotaxonomic markers as indicators of botanical identity or as tools in manufacturing can help ensure product consistency. (4) Selecting a well-characterized product suitable for testing in clinical trials will lend credibility to the findings and ensure reproducibility of studies, comparability of trials, and documentation of the weight-of-evidence. Production and evaluation of such products is impossible without validated methods accompanying reference materials.

In summary, the current research initiatives of the ODS Methods and Reference Materials Program are based on the above recommendations. The key elements of this program are (1) in the short-term, to build the infrastructure that will support the development of validated methods and reference materials; (2) in the medium-term, to fund the development of validated methods and reference materials using standard NIH mechanisms for funding projects; and (3) in the long-term, to make these methods and reference materials readily accessible to the user community. A more detailed description of these activities and the strategic focus of this program are outlined in the recently released ODS Strategic Plan. This plan can be downloaded from the ODS Web site, <http://ods.od.nih.gov/>.

References

- (1) 103rd Congress (25 October 1994) U.S. Public Law 103-417, *Dietary Supplement Health and Education Act of 1994*, <http://www.fda.gov/opacom/laws/dshea.html>
- (2) Department of Health and Human Services (2003) *Fed. Regist.* **68**, 12157–12263
- (3) Harkin, T. (2001) Senate Report 107-084, Department of Labor, Health and Human Services, and Education, and Related Agencies Appropriations Bill, 2002, pp 183–184, http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=107_cong_reports&docid=f:sr084.107.pdf
- (4) Harkin, T. (2002) Senate Report 107-216, Department of Labor, Health and Human Services, and Education, and Related Agencies Appropriations Bill, 2003, p. 153, http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=107_cong_reports&docid=f:sr216.107.pdf
- (5) Busse, W. (2000) *Drug Inform. J.* **34**, 15–23
- (6) Mahady, G.B., Fong, H.H.S., & Farnsworth, N.R. (2001) *Botanical Dietary Supplements: Quality, Safety and Efficacy*, Swets & Zeitlinger Publishers, Exton, PA, p. 20
- (7) U.S. Pharmacopeial Convention (2003) "Identification of Articles of Botanical Origin" in *The United States Pharmacopeia 26/National Formulary 21*, U.S. Pharmacopeial Convention, Inc., Rockville, MD, pp 2097–2098
- (8) Hegnauer, R. (1967) *Pure Appl. Chem.* **14**, 173–187
- (9) *Official Methods of Analysis of AOAC INTERNATIONAL* (2003) 17th Ed., AOAC INTERNATIONAL, Gaithersburg, MD
- (10) NCCAM policy on the quality of natural products, <http://nccam.nih.gov/research/policies/naturalproducts.htm>