

Challenges in Developing Analytically Validated Laboratory-Derived Dietary Supplement Databases

Joseph M Betz,¹ Catherine A Rimmer,² Leila G Saldanha,¹ Melissa M Phillips,² Karen W Andrews,³ Stephen A Wise,¹ Laura J Wood,² Adam J Kuszak,¹ Pavel A Gusev,³ and Pamela R Pehrsson³

¹Office of Dietary Supplements, National Institutes of Health, Bethesda, MD; ²Chemical Sciences Division, National Institute of Standards and Technology, Gaithersburg, MD; and ³Nutrient Data Laboratory, Agricultural Research Service, US Department of Agriculture, Beltsville, MD

Abstract

The Dietary Supplement Label Database (DSLID) is sponsored by the Office of Dietary Supplements (ODS) and the National Library of Medicine (NLM). It provides a searchable, free database of the contents of ~65,000 supplement labels. A companion database of analytically verified product labels [the Dietary Supplement Ingredient Database (DSID)] was created by ODS, NLM, and the USDA. There are considerable challenges to populating both databases, but the DSID faces unique analytic chemistry challenges. This article describes the challenges to creating analytically verified marketplace surveys of dietary supplement (DS) product content claims for inclusion in public databases. Nutritionists and public health scientists require information on actual exposures to DS constituents because labeled content may not match labeled product content. Analytic verification of composition of DSs provides a link to actual exposure. A public database of analytically derived DS content was developed to provide more accurate estimates of dietary intake in population-based epidemiologic studies. The DSID has conducted surveys of several types of vitamin- and mineral-containing DSs. Results showing label content claims as analytically derived values are available in the current DSID. A recent pilot project explored the feasibility of adding botanical DS products to the DSID. Candidates for future botanical DSID studies will be based on sales volume, potential public health impacts, and the availability of validated analytic methods and reference materials. Databases like DSID and the DSLID are essential for researchers and clinicians to evaluate dietary ingredient intakes in population-based epidemiologic studies. Together, these databases provide a picture of the DS marketplace. The DSID provides an analytic survey of marketed DSs. However, selection of future botanical supplements for DSID evaluation involves analytic challenges. Even when appropriate resources are available, method selection and data evaluation are resource- and time-consuming. *J Nutr* 2018;148:1406S–1412S.

Keywords: dietary supplement, certified reference material, label, ingredient database, laboratory analysis

Introduction

Background

The Dietary Supplement Health and Education Act (DSHEA) of 1994 (US Public Law 103-417) amended the Federal Food, Drug, and Cosmetic Act by defining a dietary supplement (DS) as any product (other than tobacco) that contains a vitamin, mineral, herb or other botanical, or amino acid intended as a supplement to the diet (1). The Act placed regulatory oversight of the category with the US FDA and defined DS as a special category of food. DS labeling regulations are incorporated in 21 CFR 101-Food Labeling (2). At the time of DSHEA's passage in 1994, there were an estimated 600 US DS manufacturers producing about 4000 products (3). A rapid expansion of the DS marketplace followed the passage of DSHEA along with a period of popular acclaim for botanical products (4, 5), propelled at least in part by a highly publicized

positive meta-analysis of St. John's wort for depression (6). The Dietary Supplement Label Database (DSLID) was launched in 2013 with funding from the NIH Office of Dietary Supplements (ODS) and the National Library of Medicine (7, 8). Since it was founded the DSLID has reflected the growth in sales and expansion of products in the marketplace. As of 2018 the database contained >65,000 labels and ~1000 new labels are added each month.

In order to evaluate the actual ingredient content in DSs, the Dietary Supplement Ingredient Database (DSID; <https://dsid.usda.nih.gov>) was developed. Its first release was in 2009. It is an analytically validated database for high-priority ingredients in DS products. For DSID studies, representative supplement products are purchased and tested by experienced laboratories for their ingredient content. The final laboratory results are statistically evaluated using weighted regression

analysis. The goals for the DSID project are to 1) establish reliable analytically predicted estimates of ingredient content in DS products; 2) compare analyzed levels of ingredients to labeled values provided by manufacturers, if available; and 3) improve dietary intake assessments by providing data files and online calculators that adjust label values into analytically predicted mean amounts. DSID estimates are based solely on the labeled level for each product category and are not brand or supplement specific. They can be used to replace labeled levels for specific DS categories to improve the accuracy of ingredient intake assessment in large epidemiologic studies. The DSID project was initiated in 2004 with studies of multivitamin/multimineral (MVM) products.

Rationale

Reliable DS- and nutrient-intake measurements have public health implications in various settings, e.g., intervention studies and the NHANES (9). For well-controlled DS intervention studies it is essential to know the identity of the raw ingredients and the quantity of nutrients and marker compounds in each serving. Clinical trials require characterization of the product integrity of nutritional and non-nutritional ingredients in the intervention materials and placebos. However, characterization of individual products is not possible for population-based estimates of intakes of nutrients and plant-derived bioactive substances. NHANES relies on accurate food and supplement composition databases to arrive at estimates of intake in a population. DS products often contain overages of labile nutrients so that products meet nutrient label claims over the shelf life of the product. The implication of this practice is that labeled content often does not match actual product. Analytic verification of claimed content in commercially sold supplements is necessary to provide this important link to consumer exposure (10). The DSID and the DSLD may be used as preliminary evaluation tools for planning intervention studies. The DSID contains mean estimates of analytic content from vetted laboratories for selected product categories and the DSLD is a transcription of product labels into a searchable database.

It is critical to recognize that the purpose, accuracy, precision, and use of the DSLD and DSID databases are different. The

contents and level of accuracy in each of the 2 databases must meet the appropriate intended uses. For example, the DSLD captures data from the label that are intended to reflect the minimum amount of a bioactive component at the end of shelf-life, within a relatively broad range of variation, and using usual scientific rounding rules that introduce further error. The DSLD is helpful for consumers who wish to learn whether a specific bioactive ingredient is contained in a product or to compare descriptions of several products on the market. It has also been used to examine claims that are made on the product. In contrast, researchers can use DSID to obtain estimates from chemical analyses of nutrients in popular types of products like multivitamin mineral supplements or omega-3 supplements compared with their label values. DSID may also be useful in a longitudinal observation study in which a crude estimate of compounds and semiquantitative intake is sufficient to categorize subjects into low to high intakes. Misuse of the databases is likely to be a problem if their origin is not understood. It is important to note that the analyzed values in DSID are representative of the products on the market at the time they were collected. No database can replace the necessity of obtaining compositional analysis for the specific lot of a supplement provided in controlled human intervention studies. For example, a human clinical intervention on a tea supplement should not use generic compositional analysis from a database, rather than analyzing the actual product consumed in the intervention. Failure to do so may be one of the underlying reasons for the heterogeneous findings in human studies of botanical supplements such as green tea. Other articles in this supplement provide examples of appropriate use and misuse of both the DSID and DSLD in more detail.

Analytical Challenges

The general purpose of analytic characterization of DSs is to generate reliable, accurate data for use in quality control (QC) by manufacturers or in enforcement actions by regulators. The challenges in analyzing DSs range from establishing the identity of the botanical source from which a product was derived to measuring the amounts of nutrients, desirable or undesirable natural and added constituents (pesticides, toxic elements, natural toxins, bioactive phytochemical marker compounds) in complex, often multi-ingredient finished products (11). DS products can contain individual nutrients, mixes of nutrients, individual botanicals, mixtures of different botanicals, and mixtures of botanicals and nutrients. They come in a variety of forms: powder, liquids, capsules, tablets, and oil-filled softgels. As a result, specialized methods may be needed to extract the ingredient(s) of interest from the product before measurements can be made (11). Surprisingly, because DS and food matrices differ, analytic methods for many micronutrients in finished DS products are often not available, even though AOAC Official Methods of Analysis (OMA) for various nutrients are available for conventional foods. The chemistry and nutrition literatures are rich in analytic procedures that have been developed and published without determining whether or not the method delivers accurate, precise, and reproducible results for their intended use. Such publications often imply, rather than prove, the correctness and validity of reported results. Peer-reviewed publications that report quantitative results for food or raw botanical ingredients but do not discuss the analytic methods in detail may not address method validity, and the methods may not be useful for commercial DS products in a different format

Supported by the NIH Office of Dietary Supplements, National Institute of Standards and Technology, and United States Department of Agriculture. Published in a supplement to *The Journal of Nutrition*. The Supplement Coordinator for this supplement was Johanna T Dwyer, Office of Dietary Supplements, NIH. Supplement Coordinator Disclosure: Johanna Dwyer is a contractor to the Office of Dietary Supplements, NIH and is employed to work in editing and preparing papers for submission; she has stock in several drug companies, some of which may sell dietary supplements, and has received partial travel and per diem costs for speaking at a symposium at the International Union of Nutritional Sciences (IUNS) meeting in a session sponsored by the International Alliance of Dietary Supplement Associations (IADSA) on content unrelated to this supplement. Publication costs for this supplement were defrayed in part by the payment of page charges. This publication must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact. The opinions expressed in this publication are those of the authors and are not attributable to the sponsors or the publisher, Editor, or Editorial Board of *The Journal of Nutrition*.

Author disclosures: JMB, CAR, LGS, MMP, KWA, SAW, LJW, AJK, PAG, and PRP, no conflicts of interest.

Address correspondence to JMB (e-mail: betzj@od.nih.gov).

Abbreviations used: DS, dietary supplement; DSHEA, Dietary Supplement Health and Education Act; DSID, Dietary Supplement Ingredient Database; DSLD, Dietary Supplement Label Database; MVM, multivitamin/multimineral; NIST, National Institute of Standards and Technology; ODS, Office of Dietary Supplements; OMA, Official Methods of Analysis; QC, quality control; SRM, Standard Reference Material; USP, United States Pharmacopeia.

or matrix than the original material analyzed. In addition, the variety of product forms and an almost infinite number of ingredient mixtures, presented in unique matrices, may create analytic interferences that are difficult to account for even when analytic methods for specific constituents exist. Reliability, accuracy, precision, and specificity are the keys to the utility of any method. However, analysts must take steps to prove that any method they use has these features for the product under consideration, especially if the method is to be used in a critical setting such as a QC laboratory, a regulatory enforcement action, or a clinical laboratory (9).

These general method requirements can be further complicated by the purpose of the analysis. At the time of the passage of the DSHEA, there were no AOAC OMA for phytochemical constituents or micronutrients in botanical or nutritional DSs. The United States Pharmacopeia (USP) likewise lacked monographs for describing and assessing botanical product quality (identity, purity, composition, strength). Furthermore, the botanical supplement industry often used methods adapted from the European Pharmacopoeia (EP) or the Deutsches Arzneibuch (DA). In much of Europe, botanical products are treated as a category of drugs, and these phytomedicines were typically simple formulations containing single herbal ingredients, and EP or DA methods were not generally suited to analysis of the complex multi-ingredient products available as DSs in the United States.

In an effort to increase the availability of methods and reference materials, the US Congress mandated the creation of the Analytical Methods and Reference Materials (AMRM) Program at the NIH ODS (12). The AMRM, along with the National Institute of Standards and Technology (NIST), USDA's Agricultural Research Service, and others have significantly increased the number of tools available to analysts. There are now 21 AOAC OMA (13) and 426 USP monographs (14) for the analysis of botanical, nonbotanical, and nutritional DSs. There are also currently 20 publicly available NIST DS Standard Reference Materials (SRMs), primarily used by researchers as tools for the validation of analytic methods and as QC materials for use when assigning values to in-house control materials (15), with another 46 in various stages of development.

Despite this progress, quantitative determination of the declared and undeclared constituents of marketed DS products remains a considerable challenge. Without a measurement structure in place it is difficult for manufacturers, researchers, and regulators to populate analytic databases and to have confidence in the numbers found in the databases.

DS Good Manufacturing Practices (GMP) require (16) manufacturers to have scientifically valid methods for verifying label claims and quantifying reasonably anticipated adulterants. For an analytic method to be considered fit for purpose, manufacturers must demonstrate the capability to deliver accurate, precise results in the presence of all components of the finished product, including fillers, tablet coatings, coloring agents, and multiple botanical and nonbotanical ingredients. Third-party analysts who are not involved in the manufacturing process have only the information provided on product labels to indicate the constituents. These analysts therefore do not always know in advance that an AOAC OMA, compendial method, published method, or in-house method will be appropriate for a specific product. Every time a new product type is tested, a method extension must be performed to establish the scientific validation of the method applied to a product. This means that a scaled-back method validation must be performed for every

new product type to ensure accurate and precise results when populating a quantitative database. When this requirement is multiplied across a broad swath of products collected as part of a large market survey, the work required can be daunting. As a result, early projects undertaken as part of the DSID focused on common nutrient product categories with mandatory nutrient labeling requirements and for which validated methods, matrix reference materials, and high-quality chemical calibrants were already available (9).

Recent Developments

Since 2004, the USDA Nutrient Data Laboratory has collaborated with the NIH ODS, the CDC's National Center for Health Statistics, and other government agencies to design and populate the DSID, an easily accessible public use database of analytically derived DS content (10). This work was initiated to fulfill the needs for reliable estimates of the vitamin and mineral content of commonly consumed DS product categories. After deployment of the nutrient studies, interactions with the DS stakeholder community identified a need to estimate exposure to bioactive supplement constituents that are not vitamins or minerals. A number of botanical products were reviewed as potential objects of the botanical project. The selection of a pilot study candidate was based on market share, availability of validated analytic methods for target analytes, and availability of high-quality matrix reference materials and calibration standards. A pilot project was conducted on green tea (*Camellia sinensis*) DSs to evaluate the feasibility of extending DSID analytic activities to include botanical ingredients and their phytochemical constituents (17).

Analytical investigations of the DSID

MVM products: methodological challenges. MVM products were chosen for the first DSID studies because these products have been shown to contribute significantly to nutrient status in population-based studies (18–20). In addition, there are numerous published analytic methods for nutrients in these products (including USP methods and AOAC OMA). An NIST SRM is available for adult MVM products and high-quality calibration standards are available for vitamins.

Although availability of these resources simplifies the analyst's tasks to some extent, the complexity of the challenge even for a relatively "simple" product category like MVMs is illustrated by the fact that the "Vitamins" heading in the Dietary Supplement Compendium of the USP contains 18 separate monographs (Table 1) (21–24). Each of the finished product monographs listed in Table 1 describes a separate type of product matrix and a distinct range of potentially interfering compounds. Furthermore, for each individual vitamin, there is a separate monograph for the raw material that includes specifications and tests for identity and strength of the vitamin. Because of this complexity, and for historical reasons, each finished product monograph offers several alternative analytic methods for each vitamin. Monograph users are required to identify, and use, a method for each nutrient in their product that is not subject to matrix interferences.

Most of the existing AOAC OMAs for vitamins are methods suitable for conventional foods (including infant formula and adult nutritional products). The methods are robust, accurate, and precise but may have limited utility for the analysis of DS tablets or capsules. A number of the OMAs are microbiological methods (examples include methods for the vitamins riboflavin,

TABLE 1 Vitamin monographs in USP 40/NF 35¹

Oil-Soluble Vitamins Capsules
Oil-Soluble Vitamins Oral Solution
Oil-Soluble Vitamins Tablets
Oil-Soluble Vitamins With Minerals Capsules
Oil-Soluble Vitamins With Minerals Oral Solution
Oil-Soluble Vitamins With Minerals Tablets
Oil- and Water-Soluble Vitamins Capsules
Oil- and Water-Soluble Vitamins Oral Solution
Oil- and Water-Soluble Vitamins Tablets
Oil- and Water-Soluble Vitamins With Minerals Capsules
Oil- and Water-Soluble Vitamins With Minerals Oral Solution
Oil- and Water-Soluble Vitamins With Minerals Tablets
Water-Soluble Vitamins Capsules
Water-Soluble Vitamins Oral Solution
Water-Soluble Vitamins Tablets
Water-Soluble Vitamins With Minerals Capsules
Water-Soluble Vitamins With Minerals Oral Solution
Water-Soluble Vitamins With Minerals Tablets

¹The Pharmacopeia of the United States of America, 40th Revision and the National Formulary, 35th Edition (14).

B-6, B-12, folate, niacin and niacinamide, and pantothenic acid), which can be problematic with DS products. For example, AOAC Official Method 2004.05, Total Foliates in Cereals and Cereal Foods (25), is a high-quality method that quantifies total folate bioactivity from several natural folate vitamers through measurements in a microbiological assay. The folate in most MVM DS products is folic acid, whose folate activity is well measured by the OMAs. However, MVM tablets and capsules are often formulated with bacteriostatic stabilizers or preservatives, which interfere with the microbiological assay (i.e., matrix interference). The alternative for MVM products is likely to be an HPLC method for folic acid. Although several of these methods are part of USP finished product monographs, there is no such AOAC OMA at present. In addition, innovative DS manufacturers are beginning to use 5-methyltetrahydrofolate in their MVM products. In the absence of bacteriostatic agents in the formulation, the microbiological AOAC OMA may be suitable, but an HPLC method developed for folic acid would not necessarily detect or quantify 5-methyltetrahydrofolate and other folate vitamers along with folic acid. Notably, some prenatal MVM supplements currently being tested for the DSID are labeled to contain both folic acid and 5-methyltetrahydrofolate.

MVM products: reference material challenges. Reference standards are used by analysts to refer to a pure chemical entity (e.g., caffeine, folic acid) to establish the identity of chromatographic peaks and to construct calibration curves for quantitative analytic methods. These can be expensive and difficult to obtain, especially with known purity. Matrix reference materials help the analyst evaluate the laboratory performance and fitness for purpose of a method. These reference materials provide quantitative values with uncertainties for target analytes in a matrix similar to that of the true test articles. NIST SRM 3280 Multivitamin/Multielement Tablets, SRM 3254 Green Tea (*C. sinensis*) Leaves, SRM 3255 Green Tea (*C. sinensis*) Extract, and SRM 3256 Green Tea-Containing Solid Oral Dosage Form were used to evaluate the method and laboratory performance of laboratories providing measurements for the DSID (26). These

materials are available through the NIST Office of Reference Materials (www.nist.gov/srm).

NIST uses several approaches for value assignment of specific analytes in SRMs. For vitamins and minerals in NIST SRM 3280, value assignment was performed through the use of a single primary method at NIST and results were confirmed by other laboratories and methods (27). The primary approach to certification of the vitamins and carotenoids at NIST is isotope dilution liquid chromatography with mass spectrometry (isotope dilution-LC-MS) (28), with a secondary method of LC with absorbance and/or fluorescence detection (29). The LC-MS approach was not commonly used at the time owing to the lack of isotopically labeled standards, so this approach to the MVM SRM analyses had to be optimized by NIST. Because of the ionization step in mass spectrometric detection, the ions in complex matrix samples may be enhanced or suppressed relative to the signal in calibrants leading to inaccurate results. The quantitative bias caused by this phenomenon can be minimized by the addition of stable isotope-labeled versions of each vitamin to the sample and the calibrants as an internal standard. In this type of isotope-dilution experiment, the internal standard and analyte of interest co-elute, correcting for inconsistencies in ionization and allowing for more accurate determination of the vitamins. In addition, MS allows for greater selectivity than absorbance detection, as specific ions (mass-to-charge ratios) of interest can be monitored for each vitamin. The combination of the improved analytic methods as well as the availability of SRM 3280 and isotopically labeled internal standards has reduced the interferences and inaccuracies in the determination of vitamins in complex samples such as multivitamins.

MVM products: DSID project. As noted, NIST SRM 3280 was used to evaluate and monitor laboratory performance for the DSID MVM studies (adult, children's, and nonprescription prenatal MVMs). Laboratories chosen for participation in the project, via federal contract or other avenues, were required to provide acceptable analytic results for samples that included this MVM SRM. Throughout the studies, results for the elements (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, zinc, chromium, and selenium) and vitamins (riboflavin, niacin, pantothenic acid, B-6, B-12, folic acid, C, A, D, and E) were compared with the NIST reference or certified values to monitor accuracy. Results with reasonable intra- and interlaboratory relative standard deviations and with a bias of <10% from the published values were considered to be acceptable. Laboratory results for other QC materials, including duplicate product samples and in-house control materials sent in every batch of commercial DSs, were also assessed as a measure of laboratory precision. If the QC results did not meet the requirements, the product samples were considered for retesting after evaluation of results for multiple lots. Through the evaluation of laboratory results from SRM 3280, the USDA could evaluate the performance of the participating laboratories and determine the quality of the measurements before finalizing the laboratory data. The results of these studies were ultimately used to predict national estimates reported in the DSID (10).

Green tea-containing products: methodological challenges. Although the analysis of the nutrient content of DSs is by no means simple, analysis of botanical DSs can be even more complicated, especially when trying to populate a relatively inclusive analytic database. In addition to the challenges

outlined already, the nature of botanical ingredients has changed significantly over the past several decades. In the past, botanical products were largely sifted, cut, or powdered plant material in the form of a tablet, capsule, tea, or tincture (12). Natural diversity in the phytochemical content of such materials was not often adjusted by manufacturers. In a relatively recent development, botanical supplement products marketed today often contain carefully controlled extracts of plant material that have been blended for phytochemical content uniformity, spray-dried onto a solid carrier or diluent, and formed into a hard or soft capsule or tablet (12). The DS GMP regulations published by the FDA in 2007 permit companies to set their own product quality specifications because there are no mandatory product composition standards (16). Extract manufacturers are free to customize extraction processes in such a way that one manufacturer's proprietary extracts derived from plant material may not (in fine detail) resemble other proprietary extracts made from the same plant. The resulting diversity in extract composition adds considerably to analytic and other scientific challenges (30). Multiple-ingredient finished products also present the analyst with the need to measure different classes of phytochemical constituents, ranging from alkaloids to flavonoids and flavonoid glycosides to polymers of various types to terpenes, in the same product. These classes of compounds can seldom be measured using the same analytic method, meaning that complete label confirmation may require the existence (or invention) of 3, 4, or more different validated analytic methods for each product.

Green tea-containing products: reference materials. NIST has developed several SRMs for analysis of botanical DSs, including a suite of green tea-containing reference materials: SRM 3254 Green Tea (*C. sinensis*) Leaves, SRM 3255 Green Tea (*C. sinensis*) Extract, and SRM 3256 Green Tea-Containing Solid Oral Dosage Form (31). Constituents of interest in green tea include monomeric, oligomeric, and polymeric catechin derivatives and methylxanthines (17). The tea leaves were *C. sinensis* leaves that were dried, ground, and sieved to 180 μm (80 mesh). A voucher specimen collected by a botanist at the green tea harvest has been stored at NIST. Half of the tea leaves were used to prepare SRM 3255, by first extracting with 50/50 (vol:vol) ethanol/water, filtering and evaporating the solvent, followed by a liquid/liquid extraction with ethyl acetate. The solvent was again evaporated, and the product was spray-dried, blended, and packaged. Finally, SRM 3256 was produced from a combination of 4 different commercially available products which had a number of additional botanical ingredients. The 4 products were ground together, sieved to 180 μm (80 mesh), blended, and packaged. The 3 materials were developed to represent the different analytic challenges associated with the determination of analytes in green tea, including extraction from different matrices and differences in concentration in the materials. The most significant challenges were related to the determination of low-level catechins, fully resolving closely related compounds, and detection of theanine (which has no chromophore). As with the multivitamin, the development of state-of-the-art separation methods for the catechins (31, 32) and the development of an ID-LC-MS method for the detection of theanine (33) were essential for the measurement community. In addition, the development of linked, authenticated materials for the leaves and the extracts allows for laboratories to develop and test identification and authentication methods.

Green tea-containing products: DSID project. The first DSID pilot botanical study evaluated the constituent content of green tea (*C. sinensis*) supplements (15). FDA requires label declarations of certain nutrients for which there is an established daily value. Declaration of individual phytochemical constituents in botanical DS products is voluntary but the amount (mass) of each botanical ingredient is required. In the green tea pilot study, most products had ≥ 1 voluntary label claim for amounts of phytochemical constituents. These claims included amounts for total polyphenols, (-)-epigallocatechin gallate (EGCG), caffeine, or total catechins. Green tea DSs were tested for 7 catechin monomers—(-)-gallocatechin, (-)-epigallocatechin, (+)-catechin, (-)-epicatechin, EGCG, (-)-gallocatechin gallate, (-)-epicatechin gallate—and caffeine. Two lots of 32 products, including capsules, powders, tablets, softgels, and liquids, were sent to 3 laboratories for analysis along with the 3 NIST green tea SRMs.

The intra- and interlaboratory precision and bias from the certified values in the SRMs were used to evaluate the quality of the data from the laboratories and determine when retesting of a particular analytic batch was necessary. The results of this study are reported in DSID-4, released in August 2017 (34).

Future Directions

A considerable amount of valuable information about the content of nutritional DSs has been provided in the DSID. Methods, reference materials, and general approaches to the population of databases for vitamin, multivitamin, mineral, multiminerals, and MVM products are now well established. Populating an analytic database for botanical DS products is likely to be more challenging owing to the variety of dosage forms and phytochemicals. For simplicity, the green tea products in the pilot study were predominantly single-ingredient green tea, but another study is underway assessing green tea constituents in mixed botanical DSs. In general, upon postdata analysis, single-ingredient products with specific voluntary label claims for catechins and caffeine were found to have higher levels than those without voluntary claims. All products provided the mandatory claim for the amount of green tea.

In the next stages of study, product dosage form integrity (ingredient release problems from coatings, lubricants, disintegrants, lot-to-lot overdrying, and overcompression) will be evaluated using USP general chapter monographs for disintegration and dissolution testing of botanicals (35). As was the case for MVM product analysis, the use of established methods and SRMs for green tea ingredients was critical for accurate evaluation of the quantitative data for use in the DSID.

Members of the ad hoc cross-agency federal working group described by Saldanha et al. (17) have selected and prioritized nonvitamin, nonmineral products for future DSID studies. Selection criteria were 1) public exposure intake from NHANES and consumer responses to commonly consumed natural products in the National Health Interview Survey; 2) public exposure sales and 5-y market trend data of individual botanicals and other dietary ingredients as reported by the most recent *Nutrition Business Journal* (36); 3) availability of validated analytic methods, such as an AOAC OMA; 4) availability of calibration standards and matrix-based certified reference materials such as a NIST SRM; 5) scientific, economic, and safety factors scored on 4 points—existence of clinical

trials, existence of epidemiologic studies, existence of safety concerns, and prevalence of known economic adulteration (15). Turmeric/curcumin DSs were chosen as the highest priority for the next round of DSID testing.

Conclusions

The establishment of databases such as the DSLD and the DSID is essential for industrial, academic, and government researchers evaluating the pervasiveness of various supplements and supplement ingredients (DSLID) in the marketplace as well as in providing a way to arrive at human exposure data to nutrients and bioactives in population-based observational studies that collect information on DS products and their patterns and frequency of use. The DSLD, although useful, only provides researchers with a searchable catalog of marketed DS products and the information provided on labels. For reasons noted earlier, labels may not provide complete information about exposure to individual bioactive phytochemicals in products and labeled nutrient content product labels may underestimate nutrient exposures. The DSID provides analytically derived quantitative estimates of DS ingredient exposure by performing analysis of products in the DS marketplace. Analyses are performed with the use of validated methods and reference materials that are fit for purpose and a defined statistical approach to sampling of marketed products and data analysis. However, generating quantitative data for DSs is analytically challenging and requires a resource-intensive and lengthy process for analyte identification, analytic method selection and performance verification, and locating suitable reference materials. Future DSID studies will continue to focus on testing ingredients and products that are commonly sold or are of interest or concern to researchers. Used in concert, the DSID and DSLD provide valuable information about the scope and nature of consumer products in the DS market.

Acknowledgments

We thank Joyce Merkel for editing and formatting and Abby Ershow for reviewing the manuscript. The authors' responsibilities were as follows—JMB, AJK, CAR, and KWA: wrote the manuscript; JMB: had primary responsibility for final content; and all authors: critically revised the manuscript and read and approved the final manuscript.

References

1. United States Public Law 103-417, 103rd Congress (October 25, 1994), Dietary Supplement and Health Education Act of 1994. Available from: <https://www.gpo.gov/fdsys/pkg/STATUTE-108/pdf/STATUTE-108-Pg4325.pdf>.
2. Code of Federal Regulations, Title 21-Food and Drugs, Chapter I-Food and Drug Administration, Department of Health and Human Services, Subchapter B-Food for Human Consumption, Part 101-Food Labeling. [Internet]. [cited 2018 Apr 17]. Available from: <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcr/CFRSearch.cfm?fr=101.9>.
3. Commission on Dietary Supplement Labels. Report of the Commission on Dietary Supplement Labels. Washington (DC): US Government Printing Office; 1997.
4. Fenyvesi C. Herbal tonic. *US News World Rep* 1998;Jan 19:70.
5. Greenwald J. Herbal healing. *Time* 1998; Nov 23:152.
6. Linde K, Ramirez G, Mulrow CD, Pauls A, Weidenhammer W, Melchart D. St John's wort for depression—an overview and meta-analysis of randomised clinical trials. *BMJ* 1996;313:253–8.
7. Dwyer JT, Saldanha LG, Bailen RA, Bailey RL, Costello RB, Betz JM, Chang FF, Goshorn J, Andrews KW, Pehrsson PR, et al. A free new dietary supplement label database for registered dietitian nutritionists. *J Acad Nutr Diet* 2014;114:1512–17.
8. NIH Office of Dietary Supplements, NIH National Library of Medicine. Dietary Supplements Label Database. [Internet]. [cited 2017 Jun 21]. Available from: <https://dslid.nlm.nih.gov/dslid/>.
9. Betz JM, Brown PN, Roman MC. Accuracy, precision, and reliability of chemical measurements in natural products research. *Fitoterapia* 2011;82:44–52.
10. Roseland JM, Holden J, Andrews K, Zhao C, Schweitzer A, Harnly J, Wolf W, Perry C, Dwyer J, Picciano M, et al. Dietary supplement ingredient database (DSID): preliminary USDA studies on composition of adult multivitamin/mineral supplements. *J Food Compos Anal* 2008;21(Suppl 1):S69–S77.
11. Mudge EM, Betz JM, Brown PN. The importance of method selection in determining product integrity for nutrition research. *Adv Nutr* 2016;7:390–8.
12. Betz JM, Fisher KD, Saldanha LG, Coates PM. The NIH analytical methods and reference materials program for dietary supplements. *Anal Bioanal Chem* 2007;389:19–25.
13. AOAC International. Official methods of analysis of AOAC INTERNATIONAL. 19th ed. 2012. [Internet]. [cited 2017 Feb 3]. Available from: <http://www.eoma.aoc.org>.
14. United States Pharmacopeial Convention. The Pharmacopeia of the United States of America, fortieth revision and the National Formulary, thirty-fifth edition. Rockville, MD: United States Pharmacopeial Convention; 2017.
15. National Institute of Standards and Technology. Standard Reference Materials. [Internet]. [cited 2017 Jul 7]. Available from: <https://www.nist.gov/srm>.
16. Food and Drug Administration. Code of Federal Regulations Title 21 Part 111. Current Good Manufacturing Practice in Manufacturing, Packaging, Labeling, or Holding Operations for Dietary Supplements; Final Rule. 2007. [Internet]. [cited 2017 Jul 17]. Available from: <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcr/CFRSearch.cfm?CFRPart=111&showFR71>.
17. Saldanha L, Dwyer J, Andrews K, Betz J, Harnly J, Pehrsson P, Rimmer C, Savarala S. Feasibility of including green tea products for an analytically verified dietary supplement database. *J Food Sci* 2015;80:H883–8.
18. Fulgoni VL 3rd, Keast DR, Bailey RL, Dwyer J. Foods, fortificants, and supplements: where do Americans get their nutrients? *J Nutr* 2011;141:1847–54.
19. Bailey RL, Dodd KW, Goldman JA, Gahche JJ, Dwyer JT, Moshfegh AJ, Sempos CT, Picciano MF. Estimation of total usual calcium and vitamin D intakes in the United States. *J Nutr* 2010;140:817–22.
20. Andrews KW, Roseland JM, Gusev PA, Palachuvattil J, Dang PT, Savarala S, Han F, Pehrsson PR, Douglass LW, Dwyer JT. Analytical ingredient content and variability of adult multivitamin/mineral products: national estimates for the Dietary Supplement Ingredient Database. *Am J Clin Nutr* 2017;105:526–39.
21. United States Pharmacopeial Convention. Dietary supplements: oil- and water-soluble vitamins with minerals capsules. In: The Pharmacopeia of the United States of America, fortieth revision and the National Formulary, thirty-fifth edition. Rockville, MD: United States Pharmacopeial Convention; 2017.
22. United States Pharmacopeial Convention. Dietary supplements: water-soluble vitamins with minerals tablets. In: The Pharmacopeia of the United States of America, fortieth revision and the National Formulary, thirty-fifth edition. Rockville, MD: United States Pharmacopeial Convention; 2017.
23. United States Pharmacopeial Convention. Dietary supplements: oil- and water-soluble vitamins tablets. In: The Pharmacopeia of the United States of America, fortieth revision and the National Formulary, thirty-fifth edition. Rockville, MD: United States Pharmacopeial Convention; 2017.
24. United States Pharmacopeial Convention. Dietary supplements: water-soluble vitamins tablets. In: The Pharmacopeia of the United States of America, fortieth revision and the National Formulary, thirty-fifth edition. Rockville, MD: United States Pharmacopeial Convention; 2017.
25. AOAC International. AOAC Official Method 2004.05, Total folates in cereals and cereal foods. 2012. [Internet]. [cited 2017 Jul 3]. Available from: <http://www.eoma.aoc.org/methods/info.asp?ID=22367>.
26. Sander LC, Sharpless KE, Wise SA, Nelson BC, Phinney KW, Porter BJ, Rimmer CA, Thomas JB, Wood LJ, Yen JH, et al. Certification of vitamins and carotenoids in SRM 3280 multivitamin/multielement tablets. *Anal Chem* 2011;1:99–108.

27. May W, Parris R, Beck C, Fassett J, Greenberg R, Guenther F, Kramer G, Wise S, Gills T, Colbert J, et al. Definitions of terms and modes used at NIST for value-assignment of reference materials for chemical measurements. NIST Special Publication 260-136. [Internet]. [cited 2017 Jul 6]. Gaithersburg, MD: National Institute of Standards and Technology; 2000. Available from: <https://www.nist.gov/sites/default/files/documents/srm/SP260-136.PDF>.
28. Phinney KW, Rimmer CA, Brown Thomas JM, Sander LC, Sharpless KE, Wise SA. Isotope dilution liquid chromatography - mass spectrometry methods for fat- and water-soluble vitamins in nutritional formulations. *Anal Chem* 2011;83:92–8.
29. Thomas JB, Sharpless KE, Yen JH, Rimmer CA. Determination of fat-soluble vitamins and carotenoids in standard reference material 3280 multivitamin/multielement tablets by liquid chromatography with absorbance detection. *J AOAC Int* 2011;94:815–22.
30. IARC Working Group on the Evaluation of Carcinogenic Risk to Humans. Some drugs and herbal products. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 108. Lyon, France: International Agency for Research on Cancer; 2016. p. 32–4.
31. Sander LC, Bedner M, Tims MC, Yen JH, Dueder DL, Porter B, Christopher SJ, Day RD, Long SE, Molloy JL et al. Development and certification of green tea-containing standard reference materials. *Anal Bioanal Chem* 2012;402:473–87.
32. Roman MC, Hildreth J, Bannister S. Determination of catechins and caffeine in *Camellia sinensis* raw materials, extracts, and dietary supplements by HPLC-UV: single laboratory validation. *J AOAC Int* 2013;96:933–41.
33. Bedner M, Sander LC, Sharpless KE. An LC-ESI/MS method for determining theanine in green tea dietary supplements. *Anal Bioanal Chem* 2010;397:1773–4.
34. US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory and US Department of Health and Human Services, National Institutes of Health, Office of Dietary Supplements. Dietary Supplement Ingredient Database (DSID) release 4.0, August 2017. [Internet]. [cited 2018 Apr 27]. Available from: <https://dietarysupplementdatabase.usda.nih.gov>.
35. United States Pharmacopeial Convention. Disintegration and dissolution of dietary supplements (2040). In: *The Pharmacopeia of the United States of America, fortieth revision and the National Formulary, thirty-fifth edition*. Rockville, MD: United States Pharmacopeial Convention; 2017.
36. Nutrition Business Journal. NBJ's supplement business report. San Diego, CA: Penton Media; 2017.