NIST/NIH Dietary Supplement Laboratory Quality Assurance Program: The First Five Exercises

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Abstract

The National Institute of Standards and Technology (NIST) has established a Dietary Supplement Quality Assurance Program (DSQAP) in collaboration with the National Institutes of Health (NIH) Office of Dietary Supplements (ODS). Program participants measure concentrations of active and/or marker compounds as well as nutritional and toxic elements in food and dietary supplement samples distributed by NIST. Data are compiled at NIST where they are analyzed for accuracy, precision, and concordance within the community. Reports and certificates of completion are sent to participants, which can be used to demonstrate compliance with current Good Manufacturing Processes (cGMPs) as determined by Food and Drug Administration (FDA). The DSQAP has conducted five exercises to date, with total participation including over 75 different laboratories and many more individual analysts.

Introduction

The dietary supplement industry in the US is booming, with 65% of adult Americans considering themselves to be supplement users (1). Consumption of dietary supplements, which includes vitamin and mineral supplements, represents an annual US expenditure of more than $25 billion (1). These figures represent an increasing American trend, as the dietary supplement industry experienced a 10-15% growth in 2009, despite a slowed economy (1). As a result, the quality and safety of these products is critically important, and must be both verified and maintained.

Reported cases of inaccurate labeling, adulteration, contamination, and drug interactions of dietary supplements have resulted in regulations that require manufacturers to evaluate the identity, purity, and composition of their ingredients and finished products. To enable members of the dietary supplements community to improve the accuracy and precision of their measurements, the National Institute of Standards and Technology (NIST) established a Dietary Supplement Laboratory Quality Assurance Program (DSQAP) in collaboration with the National Institutes of Health (NIH) Office of Dietary Supplements (ODS).

The DSQAP was originally established to improve the accuracy and precision of measurements in the dietary supplement community. In addition to measurement of active or marker compounds, the program includes nutritional elements, contaminants (toxic elements, pesticides, mycotoxins), and fat- and water-soluble vitamins in foods as well as botanical dietary supplement ingredients and finished products. The program also offers tools for demonstration of compliance with the current Good Manufacturing Practices (cGMPs) established by the US

Food and Drug Administration (FDA). The DSQAP also assists the Analytical Methods/Reference Materials (AMRM) Dietary Supplements program at the NIH in supporting the development and dissemination of analytical tools and reference materials. In the future, results from DSQAP exercises can be used by organizations such as AOAC to identify problematic matrices and analytes for which an Official Method of Analysis would benefit the dietary supplement community.

NIST has experience in the area of quality assurance programs, with longstanding programs such as the Micronutrients Measurement Quality Assurance Program (MMQAP) and the Organic Contaminants in the Marine Environment Quality Assurance Program that have been in operation since 1984 and 1987, respectively (2-8). In addition, NIST and NIH began a Vitamin D Metabolites Quality Assurance Program (VitDQAP) in 2009 (9). In contrast to the above comparability programs in which a set of analytes is measured repeatedly over time in the same or similar matrices to demonstrate laboratory performance over time, the DSQAP has a unique focus. Laboratories participating in the DSQAP do not have established, proven methodology for the samples being analyzed, nor do the samples and matrices of interest remain static over time. With a constantly changing market, these laboratories must demonstrate their performance rapidly through comparison to performance of other laboratories, and the DSQAP provides a mechanism for such intercomparison. Although the DSQAP currently functions as a survey program, some elements of the exercises such as the inorganic analytes or vitamins may have potential to evolve into a true comparability program in the future.

Experimental

Samples

Samples and analytes for each study are selected based on polling of past participants, opinions of experts, and priority within regulatory and quality control rankings. When possible, Standard Reference Materials (SRMs) developed and characterized by NIST are used as samples to ensure the highest degree of material homogeneity and certainty in the target values. If a material with a certified value is unavailable, a material may be obtained from an outside agency or prepared at NIST specifically for use in the exercise. Samples are paired with appropriate control materials whenever possible. Ideally, control materials are SRMs with certified values for the analytes of interest. The control material is selected to have a similar concentration of the analyte in comparison to the sample, and to provide comparable or fewer challenges in sample preparation. Comparison of values obtained for the control material and sample can also help identify potential analytical issues with calibration or sample preparation. Participants select the studies in which they are interested in participating for a given exercise, and samples are shipped directly to all participants. An overview of the samples used in Exercises A through E of the DSQAP is given in Table 1.

Instructions to Participants

Participants are provided with storage and stability information for all samples. Laboratories are asked to use standard in-house methods of analysis for each sample, including their own calibrants and routinely-applied methods of sample preparation, chromatographic separation, and detection. Participants are provided with the certified values for the control material when available, allowing participants to evaluate in-house analytical methodology prior to characterization of the unknown sample. Upon request, participants can also receive additional

information such as target ranges for samples, analytical methods used at NIST, and troubleshooting assistance when problems are suspected.

Data Analysis
Results from each participating laboratory are compiled at NIST approximately six months following sample distribution. Data is assessed for accuracy (when possible), precision, and concordance within the community. Certificates of participation and detailed reports, including target and consensus ranges, Z-scores, and a thorough discussion of trends, analytical challenges, and suggestions for improvement are sent to participants. Full reports, including all data tables and discussion of each study, are available on the DSQAP website (10).

Program Goals
One important goal of the DSQAP is to provide information to participating laboratories regarding overall performance with respect to measurement of nutrients, toxins, and marker compounds in various food and dietary supplement matrices. Although the DSQAP is not a proficiency testing program, information gained through participation in the DSQAP may be used by participating laboratories to demonstrate appropriateness of analytical methodology to regulatory agencies. To further improve performance, participant workshops are held to discuss results as well as methodological advancements in the characterization of dietary supplements.

Results and Discussion
Participating Laboratories
In February of 2007, four samples were distributed to 18 laboratories in the pilot exercise of the DSQAP. The fifth exercise of the DSQAP has just ended, in which five samples were distributed to 42 laboratories. In all, 75 laboratories have been involved in at least one DSQAP exercise (Figure 1). Sign-up for the sixth exercise is slated to begin in November of 2010. The program is free for participants, and laboratories are assigned identification codes for each exercise, known only to the laboratory and the program coordinators, to ensure anonymity.

Nutritional Elements
For analysis of nutritional elements in foods and dietary supplements, methods of calibration and sample preparation are critically important. Exercises A, C, and E have included nutritional element studies and have focused on these two major analytical challenges. In the pilot exercise, Ca, Mg, Fe, and Zn were determined in SRM 3280 Multivitamin/Multielement Tablets with SRM 2711 Montana II Soil as a control (11-31). Only four labs reported data, but three of the laboratories reported values within the certified range for all four elements in the multivitamin tablet sample. In nearly all cases, reported values were low for the control, where the levels for each element were two to three orders of magnitude lower than those in the sample. Most likely the low levels for the control are a result of inappropriate calibration in the range needed to accurately determine the elements at such low levels.

In Exercise C, Ca, Na, P, and Zn were determined in fortified milk powder with SRM 3244 Ephedra-Containing Protein Powder as a control (32-34). The target values for nutritional elements in the fortified milk powder were determined at NIST as part of an international interlaboratory comparison exercise. Compared to Exercise A, the sample and control were more appropriately matched in both concentration and potential matrix challenges. With 10 to

13 laboratories reporting data, the consensus data encompassed the target value, with some outliers. Upon further examination of the data for each element, a trend emerged in which laboratories reporting low values for the control also reported low values for the sample (Figure 2). The same was true for laboratories reporting high values for the control. This type of trend indicated that many laboratories may have had difficulties with calibration for all of the nutritional elements. In addition, many laboratories reported data for calcium that was much lower with respect to the true value for the control than for the sample, indicating that the analytical methods that were used did not correct for potential matrix effects. To isolate the source of these analytical problems, the similarity between the control and the sample matrices were more carefully considered.

In Exercise E, Ca, Fe, and Zn were determined in ground and flake fortified breakfast cereals of both wheat and rice origin. Participants were provided with six samples, including SRM 3233 Fortified Breakfast Cereal, ground and flake wheat cereal, ground and flake rice cereal, and a mixture of the wheat and rice flake cereals. This study was designed to investigate the participants' ability to sufficiently homogenize a sample and the effect that sampling-related inhomogeneity would have on the overall measurement uncertainty. As observed in Exercise C, the consensus data encompassed the target value for nearly all laboratories for SRM 3233, with some outliers. The same trend discussed previously was also observed in the cereal matrix, with the results of a single laboratory compared for all samples confirming the conclusion that a calibration issue is occurring in many laboratories. As a result, upcoming exercises will focus on solving the calibration problem, perhaps by inclusion of calibration materials to be measured as part of the exercise.

Contaminants

Analysis of contaminants in botanicals, dietary supplements, and foods is challenging because the levels of contaminants are often very low, approaching the detection limits of many analytical methods. In addition, the cGMPs apply to all dietary supplement manufacturers and specify the necessity of testing for contaminants and toxins in addition to active or marker compounds. Exercises A through E have all included a sample for contaminant analysis, including toxic elements in Exercise A through D and organic contaminants in Exercise E. In the pilot exercise, participants determined Pb in two dietary supplement preparations. The control tablet (SRM 3243) contained *Ephedra sinica*, and the sample tablet (SRM 3248) contained *Ginkgo biloba* (33-35). With only four laboratories reporting data, not enough information was available to draw sound statistical conclusions. Superficially, the data were scattered for both the sample and control.

In Exercise B, the sample and control were more closely matched when participants determined As in a commercial extract of *Ephedra sinica* (SRM 3242) as the control and in the aerial parts of the *Ephedra sinica* plant (SRM 3240) as the sample (33,34). The level of As in the control was about five times the level in the sample. Overall the results were very good, with the data consensus encompassing the target value for both the control and the sample with few outliers. As seen for the nutritional elements, however, laboratories reporting low values for the control also reported low values for the sample, and the same was true for those reporting high values. This trend indicates a possible calibration issue that should be addressed.

In Exercise C, the sample and control were again closely matched as participants determined As and Cd in Bitter Orange fruit (SRM 3258) as the control in a commercial extract of Bitter Orange (SRM 3259) as the sample (36). The concentration of Cd was similar in both materials, while the As level was a factor of two larger in the sample than in the control. For both toxic metals, the consensus of the results included the target value for both the sample and the control with few outliers. Of the outliers, many values for the Cd sample were surprisingly high for several laboratories, despite accurate values for the control. Upon further examination, Sn and Mo are present in higher levels in the sample than the control, which may lead to additional interferences in Cd analysis by ICP-MS for the sample matrix. Following this exercise, participants were encouraged to perform a semi-quantitative ICP-MS scan of the sample and control prior to analysis in order to identify potential interferences, and perhaps incorporate a separation method into their analytical protocol to prevent bias.

In Exercise D, the examination of Pb in *Gingko biloba* products was revisited from the pilot exercise. In this exercise, SRM 3247 *Ginkgo biloba* Extract was used as the control, while SRM 3248 Ginkgo-Containing Tablets was used as the sample (34). The concentration of Pb in the control was approximately 5 times the concentration in the sample. The consensus of the results was low and outside the target ranges for both the sample and the control, but in general most laboratories made very precise measurements of Pb in these materials. Upon further investigation, a discrepancy was identified in the moisture content of the control (~ 2 % water) and sample (~ 5 % water) materials. The target value was determined on a dry-mass basis, while the participants reported data "as-received". Following correction of the NIST certified values to "as-received" equivalents, the consensus of the data contained the target ranges and many more laboratories had reported data within the target range.

In Exercise E, an organic contaminant was investigated for the first time. Participants determined aflatoxins B_1, B_2, G_1, G_2, and total aflatoxins in SRM 2387 Peanut Butter as the control and ground peanuts as the sample (34,37,38). Ground whole peanuts were purchased from The Food and Environment Research Agency's Food Analysis Performance Assessment Scheme (FAPAS). The peanut samples were analyzed by 65 laboratories between March and April 2009 as part of the FAPAS to establish a target value for each of the aflatoxins. The levels of aflatoxins B_1 and B_2 in SRM 2387 were measured as part of an interlaboratory intercomparison exercise, while aflatoxins G_1 and G_2 were not measured in the SRM. For all aflatoxins, the concentrations in the sample and control were within a factor of two. Only five laboratories reported data, but for all compounds the reported values of all participants were within the target range. The success of this particular study indicates that the methods being used for aflatoxins analysis are sufficient, and more participating laboratories are needed to draw further conclusions.

Fat-Soluble Vitamins

The stability of fat-soluble vitamins makes their analysis challenging for even the most experienced analyst. Analysis can be complicated when vitamins are encapsulated or if the food or supplement has a high fat content or is not fortified. In addition, the weak chromophores of vitamin D and K make their determination even more difficult at the low levels found in many foods and supplements. For this reason, Exercise A was focused on determination of vitamins A and E in SRM 3280 Multivitamin/Multielement Tablets as the sample and SRM 1849

Infant/Adult Nutritional Formula as the control material (39-46). For both vitamins, the concentration in the control was two orders of magnitude lower than the concentration in the sample. Participants reported retinol values that were both higher and lower than the target value for the infant formula control, while nearly all laboratories reported values lower than the target value for the multivitamin tablet sample. These low values are likely related to the encapsulation used to stabilize retinol in the preparation of the multivitamin. In addition, retinol was present as retinyl acetate in the multivitamin and as retinyl palmitate in the infant formula, which may have resulted in calibration issues for some laboratories. Values for α–tocopherol, however, were more widely distributed for the control than for the sample, with most participants reporting values within the target range for the sample. The low levels of α–tocopherol in the control may have caused participants to use an inappropriate calibration range for the multivitamin sample. Another possibility is that some laboratories may have had experience with either multivitamin analysis or infant formula analysis, but not both, making this control/sample pair less than ideal.

In Exercise C, another study was conducted using retinol as the analyte. The sample in this exercise was a fortified milk powder, while the control was SRM 1849 Infant/Adult Nutritional Formula (40-43,45,46). The target value for retinol in the fortified milk powder was determined at NIST as part of an international interlaboratory comparison exercise. The retinol concentration in these two materials was the same, as were the potential extraction issues. Overall, the consensus of the results included the target value for both the control and the sample. The trends in the data indicate a possible calibration issue, which is common in the quantitative analysis of retinol. The concentrations of the calibration solutions are traceable to a spectroscopic absorption coefficient, not to a gravimetric value, which requires careful purity analysis on the source material. In addition, because 13-*cis*-retinol present in the sample or calibration material may bias results, separation of these isomers or appropriate calculation adjustments must be made to remove this bias.

In Exercise D, β-carotene was determined in SRM 3276 Carrot Extract in Oil as the sample and SRM 3251 *Serenoa repens* (Saw Palmetto) Extract in oil as the control (34,47,48). The level of β-carotene in the control was approximately three times the level in the sample. Both samples required minimal sample preparation as they could be diluted in organic solvent with no additional extraction required. Results from participants were significantly scattered, with values for total β-carotene ranging from 30 % to 150 % of the target value. Participants were also asked to report values for *trans*-β-carotene and *cis*-β-carotene; only half of the laboratories were able to report these values. The scatter in the total β-carotene results could be a result of improper calibration, including incorrect traceability as discussed above for retinol or presence of β-carotene isomers for which concentrations were incorrectly accounted.

In Exercise E, β-carotene determination was revisited using SRM 3280 Multivitamin/ Multielement Tablets as the sample and SRM 3251 *Serenoa repens* (Saw Palmetto) Extract in oil as the control (48). The amount of total β-carotene in the sample was approximately 10 times the amount in the control material. The extraction of the β-carotene from the sample was significantly more challenging than from the control material. Most laboratories reported moderate uncertainties for total β-carotene, and the consensus of the results fell within the target range. One group of laboratories reported correct values for the control material but low values for the sample, with another group reporting correct values for the sample and high values for the

control. This type of trend again points to a calibration issue. Less than half of the participating laboratories reported values for the isomers of β-carotene, again indicating that many laboratories do not have the capability to measure each isomer specifically, which may contribute to the observed calibration issues. Another consideration is that the difference in matrices between the sample and control may have led some laboratories to use an improper extraction procedure for the sample, in which β-carotene is encapsulated. In addition, isomerization or degradation of β-carotene may occur during many extraction procedures. Another concern was the uncertainty and potential errors associated with the spectrophotometric determination of calibrant concentration for β-carotene. In future exercises, participants could be provided with a molar absorptivity for β-carotene and be asked to use this value in addition to the in-house value when calculating final concentrations for comparative purposes. Participants should also be informed more completely as to the nature of the analyte within the matrix and future control samples will be better matched with regard to extraction challenges.

Water-Soluble Vitamins

Compared to analysis of fat-soluble vitamins, the analysis of water-soluble vitamins has significantly fewer potential issues. Most of these vitamins are relatively stable and are fortified at high levels in foods and dietary supplements of interest to the DSQAP. In the pilot exercise, participants were asked to determine the level of folic acid in SRM 3280 Multivitamin/ Multielement Tablets as the sample and SRM 1849 Infant/Adult Nutritional Formula as the control (49-51). The level of folic acid was 200 times greater in the sample than in the control. The consensus of the reported data included the target value, although results were scattered for the lower-level control. This scatter is likely the result of a calibration issue, in which the level of folic acid in the control was out of the linear range used for calibration of the method.

In Exercise B, participants analyzed SRM 3280 Multivitamin/Multielement Tablets for thiamine and riboflavin content without a control material. For both vitamins, the consensus of the data contained the certified values measured by NIST (51,52). The spread in the data for both vitamins was greater than expected, with values ranging from 60 % to 180 % of the certified value for thiamine and from 70 % to 170 % of the certified value for riboflavin. Without a control material, the results from this exercise are difficult to interpret, but further investigation into the ability of DSQAP participants to accurately measure these compounds is necessary.

In Exercise C, the measurement of two different water-soluble vitamins was investigated. Participants determined the concentration of niacinamide and pyridoxine in a fortified milk powder as the sample and in SRM 3244 Ephedra-Containing Protein Powder as the control (33,34). The target values for niacinamide and pyridoxine in the fortified milk powder were determined at NIST as part of an international interlaboratory comparison exercise. The level of niacinamide in the control was approximately 3 times the level in the sample, while the level of pyridoxine in the control was approximately 2.5 times the level in the sample. For niacin, the results for the sample and control were more scattered than expected, and the scatter could not be correlated to the analytical method used. The scatter in the niacin sample spanned a significant range, from less than 20 % to more than 500 % of the certified value in the sample. The results for pyridoxine were also more scattered than expected, and as with niacinamide, the scatter was not correlated to the type of analytical method used. For both vitamins, the consensus of the reported data did encompass the target value, however the large amount of scatter was a concern.

One concern was that the participating laboratories reported niacin (nicotinic acid) instead of niacinamide or pyridoxine instead of pyridoxine hydrochloride. Another possibility is a calibration issue, which should be investigated further in future exercises with a more closely matched sample and control.

In Exercise D, the measurement of niacinamide was revisited with fortified milk powder as the sample and SRM 1849 Infant/Adult Nutritional Formula as the sample (41,53). The target value for niacinamide in the fortified milk powder was determined at NIST as part of an international interlaboratory comparison exercise. The level of niacinamide in the control is very similar to the level of niacinamide in the fortified milk powder. To eliminate the possibility of calibration issues, a vial containing 500 mg of USP niacinamide was provided to all participants for use in method calibration. Compared with the results from Exercise C, the results in Exercise D were significantly improved. The consensus of the data included the target value and few laboratories reported significantly outlying data. The results from this exercise highlight the importance of proper selection and use of calibration materials. Reference materials must be appropriate (e.g., niacinamide rather than nicotinic acid) and should be screened for purity for increased measurement comparability.

In Exercise E, niacinamide was measured again in a very different but well-matched sample and control set. The control for this study was SRM 3233 Fortified Breakfast Cereal, and the sample was a ground breakfast cereal containing a similar concentration of niacinamide. The target value for niacinamide in the ground breakfast cereal was determined at NIST parallel to the certification of water-soluble vitamins in SRM 3233. The consensus of the reported data included the target value for both the sample and the control, with few outliers. A comparison of the values that were reported for the sample and control from each laboratory indicated a potential calibration issue, as discussed and solved in Exercise D. This again stressed the necessity of using well-characterized control materials in all measurements, not only when specifically asked to do so. Because participants performed so well on the niacinamide study of Exercise E, future exercises will consider more complex matrices containing lower, non-fortified levels of niacinamide or perhaps sample and control pairs that are less exactly matrix matched. In addition, future exercises will be planned to expand to other water-soluble vitamins, building on the knowledge gained through this series of niacinamide studies.

Botanicals

The analysis of botanical dietary supplements is incredibly challenging, as by definition these materials are complex natural products. In addition to the complexity of the sample matrix, reference standards are often unavailable for many of the compounds of interest. When reference standards are available, purity is often insufficient for true quantitative analysis, as the standards are often isolated from the plant materials and include isomers and other related compounds. A further complication lies in the lack of well-developed methods or a consensus of the community regarding the best analytical approach for new materials. The DSQAP has focused several exercises on addressing some of these analytical challenges.

In the pilot exercise, participants measured caffeine in SRM 3260 Bitter Orange-Containing Solid Oral Dosage Form as the sample and SRM 3243 Ephedra-Containing Solid Oral Dosage Form as the control (33,54,55). The consensus of the reported data included the target value for

both the sample and the control, with few outliers. Because participants performed so well on the analysis of caffeine, future exercises will involve measurement of more complex botanical analytes.

Exercise B included three botanical analytes: synephrine, flavonols, and phytosterols. Participants measured synephrine in SRM 3258 Bitter Orange Fruit as the sample and in SRM 3259 Bitter Orange Extract as the control (56). The level of synephrine was approximately ten times greater in the control than in the sample. Overall the consensus of the data for synephrine contained the target value for both the control and the sample, with few outliers. The results for synephrine in the sample and control were slightly low for all participants, indicating a potential extraction or calibration issue. Extraction issues are common for botanical matrices when the compound of interest must be extracted from the natural environment within the plant tissues. Participants in Exercise B also measured flavonols quercetin, kaempferol, and isorhamnetin in SRM 3248 Ginkgo-Containing Tablets as the sample and SRM 3247 *Ginkgo biloba* Extract as the control (34,57-59). The level of each flavonol was approximately 5 to 10 times greater in the control than in the sample. For all three compounds, the consensus of the data contained the target value for both the sample and the control, with few outliers. As with synephrine, a potential calibration issue was apparent. Lastly, participants determined the levels of phytosterols campesterol, β-sitosterol, and stigmasterol in SRM 3250 *Serenoa repens* (Saw Palmetto) Fruit as the sample and SRM 3251 *Serenoa repens* (Saw Palmetto) Extract as the control (48). The level of each flavonol was approximately 5 to 10 times greater in the control than in the sample. For each of the phytosterols, only five laboratories reported data. The data that was reported, however, was very scattered and the consensus of the data contained the target value for only one of the compounds (stigmasterol). Because the data was scattered and a limited number of laboratories reported data, further studies on phytosterols analysis would be planned in future exercises.

The study in phytosterols analysis from Exercise B was repeated in Exercise C with the inclusion of a phytosterol solution. Participants measured campesterol, β-sitosterol, and stigmasterol in SRM 3250 *Serenoa repens* (Saw Palmetto) Fruit as the sample and SRM 3251 *Serenoa repens* (Saw Palmetto) Extract as the control (48). At NIST, a solution of mixed phytosterols was gravimetrically prepared in chloroform at a concentration that would be appropriate for a calibration, requiring only derivatization prior to analysis. The participants accurately measured the concentration of each of the phytosterols in the solution, although the measurements were less precise than expected. Participant questionnaires indicated that most laboratories utilized in-house methodology involving a hydrolysis step that was unnecessary for a simple solution. The additional sample processing associated with the hydrolysis step may lead to the increased uncertainty. Some participants also reported an inability to weigh the solution reproducibly, likely a result of solvent evaporation, which may have also contributed to the increased uncertainty. Most laboratories accurately determined the levels of the phytosterols in the control material, SRM 3251 *Serenoa repens* (Saw Palmetto) Extract, which required hydrolysis and derivatization prior to analysis. Comparatively, most laboratories reported values significantly lower than the target values for the phytosterols in the sample, SRM 3250 *Serenoa repens* (Saw Palmetto) Berries, which required extraction, hydrolysis, and derivatization prior to analysis. Taken together, these results indicated that the extraction of the phytosterols from the fruit was incomplete and additional successive extraction steps may be necessary for quantitative analysis.

In addition, most laboratories reported the addition of an internal standard immediately prior to the derivatization step, whereas ideally the internal standard would be added prior to the extraction step in order to reduce uncertainty and improve accuracy.

In Exercise D, participants measured organic acids (citric acid, malic acid, and quinic acid) in SRM 3291 *Vaccinium myrtillus* (Bilberry) Extract as the sample and SRM 3281 *Vaccinium macrocarpon* (Cranberry) Extract as the control (60). The level of each acid was within a factor of two between the sample and the control material. For quinic and malic acids, the consensus of the reported data included the target value for the sample, while the reported data for citric acid was more scattered. The consensus ranges for each acid were very small, indicating excellent overall precision. Surprisingly, the uncertainty for the determination of organic acids was greater in the control material than in the sample. Several laboratories reported chromatographic interferences, particularly for quinic acid, in the control material that did not affect analysis of the sample. In addition, several laboratories reported low values for both the sample and the control material which may be related to the use of solid-phase extraction for sample cleanup. A recommendation was made to participants to investigate recovery by using a calibration material prior to using a filter or solid-phase extraction cartridge for quantitative analysis.

In Exercise E, participants measured eight catechins (catechin, epicatechin, epicatechin gallate (ECG), epigallocatechin (EGC), epigallocatechin gallate (EGCG), gallocatechin (GC), and gallocatechin gallate (GCG)) in SRM 3256 Green Tea-Containing Tablets as the sample and SRM 3255 *Camellia sinensis* Extract as the control. While participants were asked to report values for all of the catechins, not all chromatographic methods can separate all of the catechins, nor were calibration standards readily available for each of the analytes. Overall, the results for all catechins in both the sample and the control material were extremely scattered. Relative to the values reported for the sample, the values for the control material appeared to be less scattered, indicating a possible bias in the extraction step. One bias might be from epimerization occurring during the extraction process. When the reported values for each laboratory were summed to determine a value for total catechins, the results were much less scattered and the consensus range of values was aligned with the target range, supporting the explanation of epimerization. In addition, laboratories and regulators might be more interested in a value of total catechins for labeling and quality control than individual values for each catechin.

Fatty Acids

For the determination of fatty acids in foods, several sample preparation steps are required. A common approach involves transesterification of the existing triglycerides for analysis as fatty acid methyl esters. Although this procedure is well established and routine in many food and dietary supplement laboratories, the labor intensive sample preparation can lead to issues with calibration and increased uncertainty. In the pilot exercise, participants measured fatty acids in SRM 3278 Tocopherols in Edible Oils as the sample and SRM 3276 Carrot Extract in Oil as the control (34,47). Only four laboratories participated in this study, making statistical analysis of the data difficult. Three of the four laboratories reported data that correlated well with the target values for each fatty acid as well as for total fatty acids. One laboratory was an outlier for every analysis, reporting values that were significantly lower than the target and consensus for each fatty acid and the total fatty acids. The results from this study were promising, and the analysis

of fatty acids was planned for a future study with the hope that more laboratories would participate.

In Exercise C, participants measured fatty acids in SRM 3274-1 Botanical Oils Containing Omega-3 and Omega-6 Fatty Acids (Borage, *Borago officinalis*) as the sample and SRM 3274-2 Botanical Oils Containing Omega-3 and Omega-6 Fatty Acids (Evening Primrose, *Oenothera biennis*) as the control. For linoleic acid and γ–linolenic acid, the consensus of the reported data included the target value for the sample, while the reported data for α-linolenic acid was significantly lower than the target value for the sample. Because the consensus values for two of the three fatty acids of interest were acceptable while the value for the third was not, the α-linolenic acid in these oils may be degrading. A comparison of the data from each laboratory for linoleic acid and γ–linolenic acid indicated that laboratories reporting low values for each fatty acid with respect to the target value in the control material also reported low values with respect to the target value in the sample. The same was true for laboratories reporting high values with respect to the target values. This type of correlation indicated a potential issue in the derivatization process or in the calibration procedure. As investigated with the phytosterols, inclusion of a calibration solution for fatty acid studies in future exercises may aid in troubleshooting problems of this nature.

Conclusions

Through five exercises of the DSQAP, many lessons have been learned by both the participants and the program coordinators. Overall, laboratories have performed as expected on most of the studies. When analytical issues were identified, they were attributed to common analytical issues such as incomplete extraction or improper calibration. The DSQAP has identified these problems and encouraged participants to use these lessons to improve their measurement capabilities.

In several cases, follow-up studies have directly identified an analytical issue and provided an insight into the real challenges facing analytical laboratories in the dietary supplement community. First, incomplete extraction and addition of unnecessary analysis steps complicated the analysis of phytosterols in botanical oils. Troubleshooting of this problem involved eliminating potential matrix issues and providing participants with a calibration solution for comparative analysis. Second, participants demonstrated poor overall measurement precision and accuracy for determination of niacinamide in infant formula. The study was repeated with inclusion of a common reference standard for calibration, and results improved significantly, demonstrating the need for complete characterization of all reference standards. Additionally, the analysis of lead in botanicals demonstrated the importance of moisture consideration in a reported value. Moisture present in the material being analyzed as well as in reference standards can affect determination of both organic and inorganic analytes and should always be considered.

Other common analytical problems have been discovered through all of the exercises and all of the studies. Some laboratories have incorrectly used the target value provided for the control material to determine a scaling factor for analysis of an unknown material. The difference in matrices between and control and sample are often different, making a mathematical adjustment for recovery invalid. The target range for the control is provided only as a quality self-check. For organic analytes, recovery issues as a result of loss upon filtration or sample clean-up have

been identified. Participants are encouraged to test all such filters and cartridges for recovery using a calibration solution to identify potential sources of loss and subsequent measurement bias.

A future goal of the DSQAP is to identify upcoming problems and challenges in the dietary supplement community. The nature of regulation in the food and dietary supplement industry leaves the analytical methodology several steps behind in emerging markets. When new products and matrices are introduced to the market, the DSQAP hopes to organize exploratory exercises to help laboratories demonstrate performance within the community in the absence of a target value. These DSQAP studies can also identify analytes for which high-quality reference standards are needed, identify matrices for which reference materials are needed, and assist groups such as the AOAC in determining for which analytes and matrices official methods of analysis are needed.

As the DSQAP evolves, participants are polled regularly to identify types of samples and analytes that are of emerging interest. The variety of participants, from government laboratories to third-party analytical laboratories to dietary supplement manufacturers, leads to differing priorities and a continuing need for participant involvement and feedback. Additional guidance from regulatory agencies and trade associations assists the DSQAP coordinators in maintaining a relevant and useful program.

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Table 1. Summary of analytes and samples used in DSQAP Exercises A through E.

	Exercise A 2007	Exercise B 2008	Exercise C 2008	Exercise D 2009	Exercise E 2010
Nutritional Elements	**Ca, Mg, Fe, Zn** Montana Soil Multivitamin Tablets		**Ca, Na, P, Zn** Chocolate Energy Drink Infant Formula		**Ca, Fe, Zn** Breakfast Cereals
Contaminants	**Pb** Ephedra Tablets Ginkgo Tablets	**As** Ephedra Aerial Parts Ephedra Extract	**As and Cd** Bitter Orange Fruit Bitter Orange Extract	**Pb** Ginkgo Extract Ginkgo Tablets	**Aflatoxins** Peanut Butter Peanuts
Fat-Soluble Vitamins	**Retinol, α-tocopherol** Infant Formula Multivitamin Tablets		**Retinyl palmitate** Infant Formulas	**β-carotene** Saw Palmetto Extract Carrot Extract in Oil	**β-carotene** Saw Palmetto Extract Multivitamin Tablets
Water-Soluble Vitamins	**Folic Acid** Infant Formula Multivitamin Tablets	**Thiamine, Riboflavin** Multivitamin Tablets	**Niacinamide, Pyridoxine** Chocolate Energy Drink Infant Formula	**Niacinamide** Reference Standard Infant Formulas	**Niacinamide** Breakfast Cereals
Botanicals	**Caffeine** Bitter Orange Extract Bitter Orange Tablets	**Flavonols** Ginkgo Extract Ginkgo Tablets	**Phytosterols** Phytosterol Solution Saw Palmetto Extract Saw Palmetto Fruit	**Organic Acids** Cranberry Extract Bilberry Extract	**Catechins** Green Tea Extract Green Tea Tablets
		Phytosterols Saw Palmetto Extract Saw Palmetto Fruit			
		Synephrine Bitter Orange Extract Bitter Orange Tablets			
Fatty Acids	**Fatty Acids** Carrot Extract in Oil Vegetable Oil Blend		**Ω3, Ω6 Fatty Acids** Borage Oil Evening Primrose Oil		

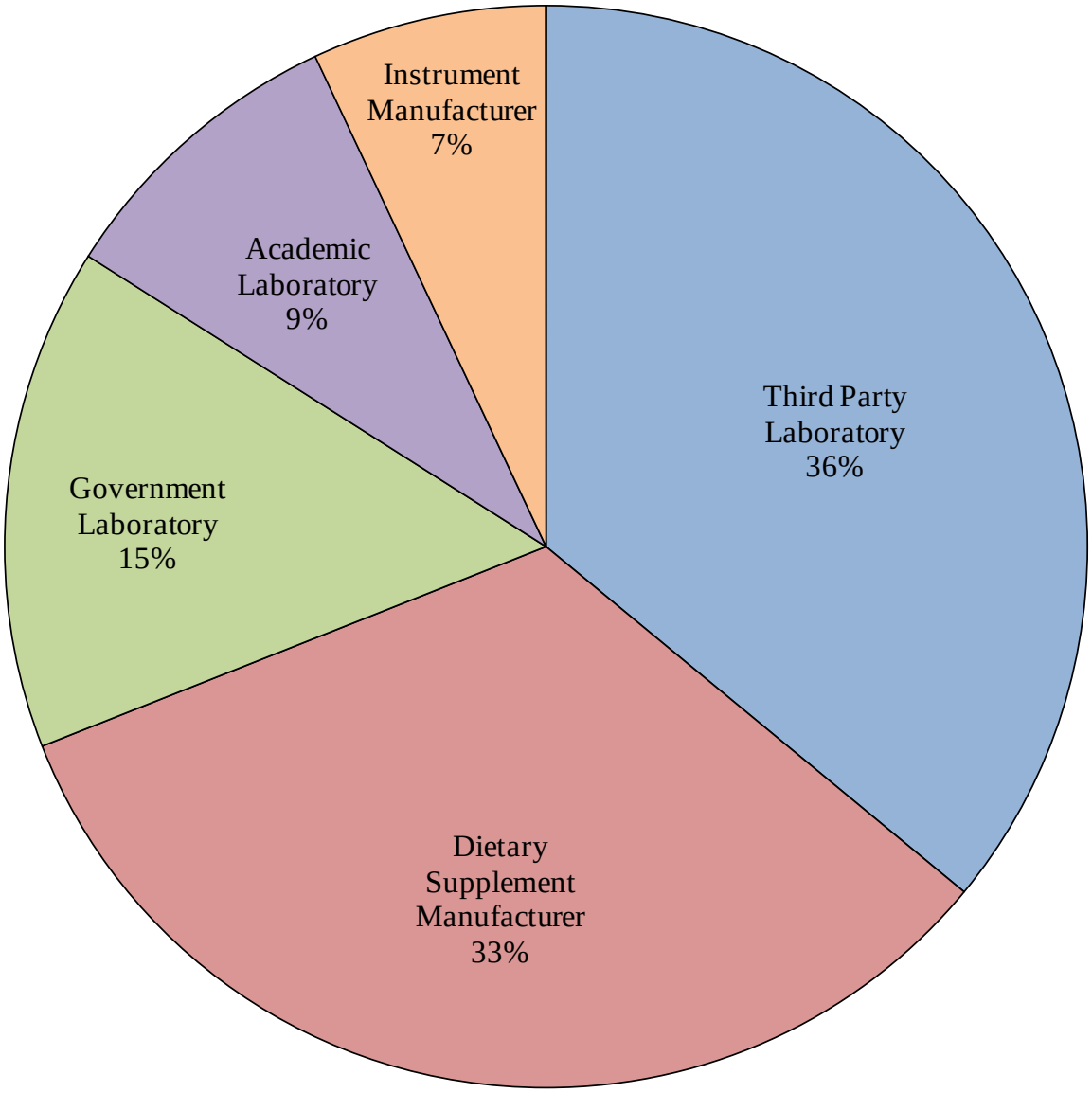

Figure 1. Distribution of participants in Exercises A through E of the DSQAP. In total, 75 laboratories have participated in at least one exercise.

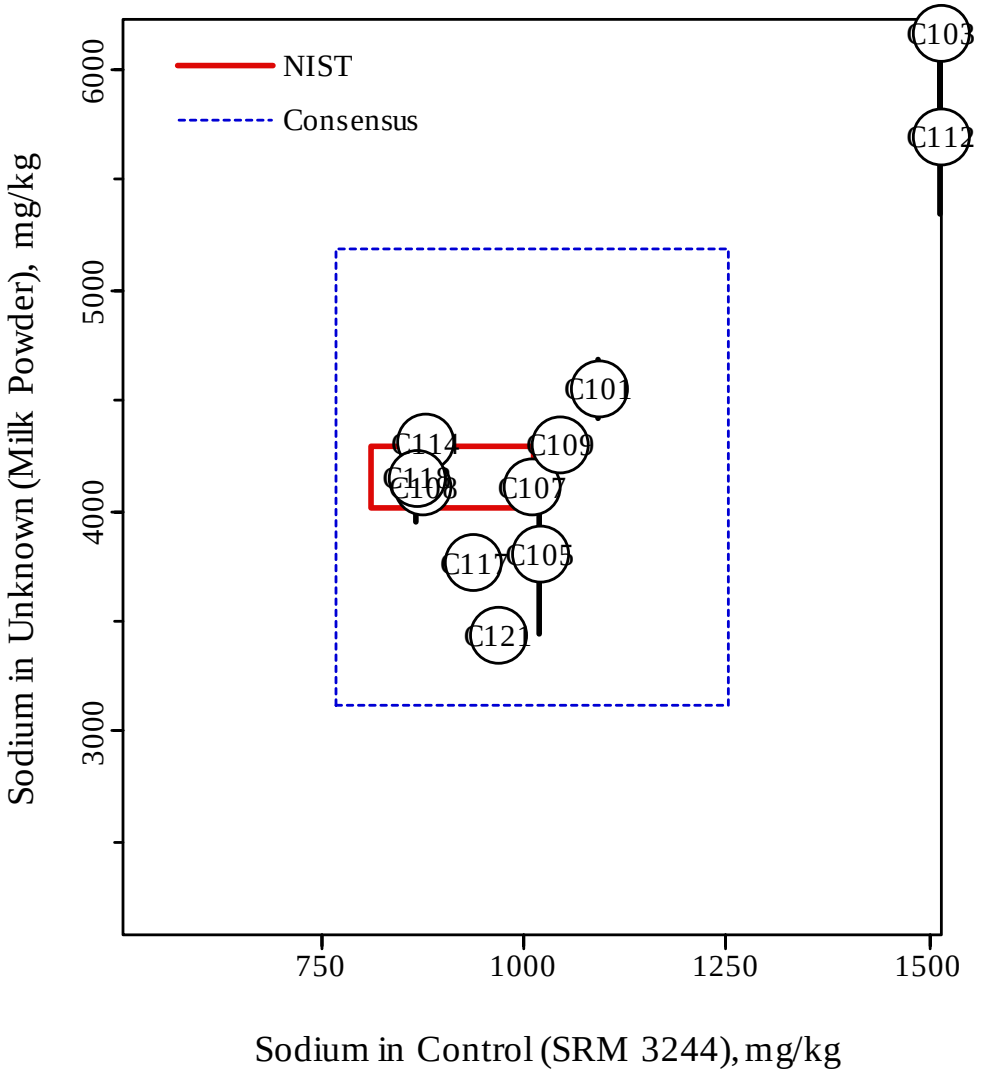

Figure 2. Comparison of results from Exercise C for determination of sodium in fortified milk powder with SRM 3244 Ephedra-Containing Protein Powder as a control. This type of trend may indicate difficulties with calibration for Na, as well as the other nutritional elements in this exercise. This trend was also observed for many other analytes in the various exercises.