GMPs: The Other Pieces of The Puzzle

NIST continues to learn valuable lessons about the challenges facing the dietary supplement community.

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In the November 2010 issue, this column described the GMP regulation as a puzzle. With help from NIST colleagues, one piece of the puzzle was described—a program that exists to help labs demonstrate their competence. As it turns out, NIST has even more programs that can assist this industry in achieving GMP compliance.

Frequently overlooked in discussions of the dietary supplement GMPs is the level of trust that FDA placed in the industry's ability to manufacture high-quality products. The government was not prescriptive in the GMP. They did not dictate the standards and tests that this large and highly diverse industry would have to use for assuring their products were safe and of high quality. Individual manufacturers are required to *set their own specifications* and to test against specifications. Again, FDA didn't tell companies how to test, it just laid out simple ground rules with descriptors like "scientifically valid" and "suitable for intended use."

With trust comes great responsibility. If the government tells you that you must do "something" but does not say exactly what that "something" is, then you're going to have to figure it out yourself. In that context, the more options you have that allow you to fulfill the mandate, the better off you are. In past columns different sets of tools have been discussed, such as analytical methods validation, materials, and test methods for identification and chemical calibration standards. One very nifty tool in the dietary supplement analytical toolbox is **matrix reference materials**, which can aid in the development of methods and support **laboratory performance**.

Previously the Dietary Supplements Laboratory Quality Assurance Program (DSQAP) run by NIST was introduced as providing an overview of relative performance within the dietary supplement laboratory community. In truth this program can provide much more than training and performance overview, so I will now turn the column over to my colleagues at NIST to explain how to get the most out of this unique (and free!) program.

The NIST Program for Dietary Supplements

The constantly changing dietary supplement market and the high degree of innovation that creates an enormous diversity of finished products makes repeated determination of a few target compounds in a single matrix of little use to participants. The wide range of matrices and

analytes under the “dietary supplement” umbrella means that not every laboratory is interested in every sample or analyte. Instead, participating laboratories are interested in testing in-house methods on challenging, real-world matrices to demonstrate that their performance is comparable to that of the community. They aren’t interested in analyzing, say, a ginseng product if they’re not in the business of manufacturing ginseng products, and they need to know that they’re getting the right answers when they analyze their own products. In an area where there are few standard methods, the DSQAP offers a unique tool for assessment of the quality of measurements and can provide feedback to labs that lets them know how well they are performing. Importantly, it can also help them troubleshoot and improve their operations.

For every analyte and matrix of the DSQAP exercises conducted to date, statistical outliers have been identified in the data. For some analytes and matrices, more laboratories were outliers than were in consensus! While on the surface this can be upsetting for those laboratories involved, in the overall scheme of the DSQAP, these exercises offer the most important information to the dietary supplement community—a chance to identify and resolve analytical problems from a large set of independent data.

Take for example the analysis of saw palmetto materials. In this exercise, participants were asked to measure phytosterols in saw palmetto berries as well as in saw palmetto extract, which is an oil. Measurement of phytosterols in saw palmetto extract by GC-MS requires only that hydrolysis and derivatization be performed, while analysis of berries requires an extraction step, followed by the hydrolysis and derivatization. The results of the first saw palmetto exercise were widely scattered and the consensus values were significantly lower than expected. The methodological information provided by participants did not elucidate the cause of problem.

In the subsequent exercise, participants were sent the same two saw palmetto products, as well as a solution containing the three phytosterols in an appropriate solvent – the idea being that the solution would require only derivatization, removing one level of complication (the hydrolysis) from the analysis. The results from the second exercise were no less scattered, but based on the methodological information, it was clear that laboratories were performing all three sample preparation steps on all of the materials in an effort to use consistent practices for all products. Theoretically, this is an analytically appropriate approach to phytosterol analysis; however, use of unnecessary sample preparation steps was shown to increase variability in the measurement. In spite of the increased variability, it was evident that most laboratories had properly performed the derivatization and that the most significant source of low values in the berry material was due to incomplete extraction. Several recommendations were offered to participants, including adaptation of sample preparation procedures based on prior knowledge of the sample.

Another interesting problem was seen in the analysis of lead (Pb) in ginkgo leaves and extract. In general, results from elemental analysis studies have a much tighter consensus range than the studies of organic compounds like the phytosterols discussed above. The results for lead in both the control and the unknown sample showed both within- and between-laboratory precision.

However, there was an apparent discrepancy between the consensus values achieved by the participating laboratories and the target values, representing the NIST certified values. After a closer look, the difference between the consensus and target values corresponded to the amount of moisture in the ginkgo materials! The NIST certified values are reported in the Certificates of Analysis as "dry-mass" basis, indicating that they have been corrected for some amount of water content determined at the time of certification. Participants, however, had been asked to report lead values "as-received." This provided a concrete example of the importance of reading the fine print. When using a certified reference material as a quality control material, results must be compared on the same basis – either as-received or dry mass, depending on how the values are expressed in the Certificate of Analysis.

For a final example, consider the analysis of niacinamide in infant formula. Niacinamide was chosen as a test case because it is water-soluble, easy to extract, and stable compared to other vitamins. In addition, the fortified levels in infant formula are relatively high at around 100 µg/g. The results of the first niacinamide study were shocking—participants reported values ranging from 5 µg/g to 500 µg/g! The methodological information provided by participants did not indicate that the extraneous data were correlated with a specific type of analysis (i.e., microbiological assay or LC determination).

In the subsequent exercise, the same infant formula sample was distributed, accompanied by a common reference standard (500 mg USP niacinamide, donated by the USP). The reported range for the second study was 90 µg/g to 150 µg/g, and if the maximum and minimum reported values are excluded, the range tightens to 100 µg/g to 115 µg/g. In addition, the consensus range was contained within target range—an ideal situation for the DSQAP. The take-home message here is that characterization of the chemical reference standards used in calibration is of utmost importance. When reference standard purity is removed from the equation, as demonstrated here by having all participants use a common, high-quality chemical reference material, the consensus range is much tighter.

Through the first five exercises, NIST staff and participants have both learned valuable lessons about the challenges facing the dietary supplement community. Based on participant recommendations, future exercises will include a wider variety of samples to allow participants to demonstrate their comparability to other laboratories in the community.

Editor's Note: *To get your laboratory involved in the DSQAP, send an email to DSQAP@nist.gov; you will be added to the mailing list for notification of upcoming exercises and events. For more information or to see reports from past exercises, visit the website at http://www.nist.gov/mml/analytical/dsqaprogram.cfm.*