

Assessment of Vitamin D in Multivitamin/Mineral Dietary Supplements

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Abstract

Vitamin D is a nutrient of public health concern and is naturally present in some foods, added to others, and available in dietary supplements. It is essential for bone growth and may have other roles in human health. To estimate current levels of intake, analytical data for vitamin D in foods and dietary supplements are required.

The Nutrient Data Laboratory, in conjunction with the National Food and Nutrient Analysis Program and the NIH/Office of Dietary Supplements, has been working with a contracted laboratory to develop a method of analysis for vitamin D in dietary supplements. The HPLC method under development uses [H-3]-cholecalciferol as an internal standard, and quantifies both ergocalciferol and cholecalciferol. Using this method, results for the National Institute of Standards and Technology Standard Reference Material (NIST SRM) 3280, a multivitamin/mineral (MVM), currently average 2% below the certified value with a relative standard deviation (RSD) of 6% (n=10). Two MVM products have been developed as in-house control materials (RSDs of 8 and 14%). Blind duplicates (2 sets of 20 tablets of the same MVM product) were sent to the lab with each analytical batch. These duplicate results (calculated by percent difference from the mean) ranged from 1-11% (n=15), and 17-19% (n=2). This method is being applied to the analysis of vitamin D in MVM products for the Dietary Supplement Ingredient Database.

Introduction

The Nutrient Data Laboratory (NDL), Beltsville Human Nutrition Research Center (BHNRC), Agricultural Research Service (ARS), USDA, has been working with the Office of Dietary Supplements, National Institutes of Health (ODS/NIH) and other federal agencies to plan and develop the Dietary Supplement Ingredient Database (DSID) to evaluate levels of ingredients in dietary supplement products. The DSID is funded, in large part, by ODS.

NDL recently sampled and analyzed adult and children's MVM products to determine levels of 18 nutrients. The results for 18 nutrients were released as the Dietary Supplement Ingredient Database, Release 1 (DSID-1).

Preliminary results were obtained for Vitamin D in adult MVM products before the National Institute of Standards and Technology Standard Reference Material (NIST SRM) 3280 certified values were finalized. When the NIST SRM 3280 results were evaluated, they were consistently low (approx. 20%). Additional validation of the method of analysis for vitamin D was required and a lab was contracted to develop a more accurate method.

NIST SRM 3280 was used to develop and validate the method. Along with the NIST SRM 3280, three MVM products were developed as in-house control materials, one adult and two children's products. Developing in-house control materials are essential due to the high cost and limited availability of the NIST SRM 3280. It is also necessary to account for the multiple matrices available in supplement products.

Methods and Materials

Adult and children's MVM products included several different sample matrices. To optimize the new method of analysis for vitamin D sample handling, batch preparation, and quality control protocols were established.

Sample Handling

- 20 units (tablets, powders) of each sample were weighed and homogenized.
 - Soft gels were weighed, emptied, rinsed, and then the shells were reweighed for the calculation of the weight of the sample.
 - Liquids were weighed in 20 ml aliquots with the specific gravity reported.
 - Tablets and caplets were weighed and homogenized in a bone grinder.
 - Gummies were dissolved in water and gumballs were dissolved in a solvent for homogenization.

- After homogenization, samples were stored in a freezer at -30°C.

The lab was given general information about each sample matrix, the vitamin D form, and the approximate range expected.

Method of Analysis

For the analysis of Vitamin D:

- Each sample was aliquoted and [³H]-Cholecalciferol was added as a standard to estimate recovery.
- Samples were digested for 1-2 hours at 60°C with methanolic KOH.
- Water was added to the samples after which they were extracted 3 times with hexane/ethyl acetate solution. The extractions were combined and concentrated under vacuum, and then suspended in a hexane/methylene chloride solution.
- The concentrated samples were applied to a silica SPE column.
- The vitamin D fraction was eluted, dried under vacuum and applied to a semi preparative (0.9 x 25 cm) Zorbax Sil HPLC column.
- This vitamin D fraction was collected, dried and chromatographed using a Zorbax NH2 analytical HPLC column.
- Final quantitation and separation of ergocalciferol and cholecalciferol was achieved with an Altech C18 column.
- The vitamin D was estimated by comparing peak heights of unknowns to known amounts of Cholecalciferol standards followed by correction for recovery with [³H]-Cholecalciferol.

Batch Preparation and Quality Control

Multivitamin/mineral dietary supplement products were purchased, repackaged and prepared in batches to send for laboratory analysis. Each batch contained 20 samples which included the following:

- 1 Certified Reference Material (NIST SRM 3280)
- 2 In-house Control Materials
- 2 Blind Duplicate Samples
- 15 Individual test samples

Each sample was homogenized and analyzed with the other samples from the same batch, to monitor precision and accuracy.

Figure 1

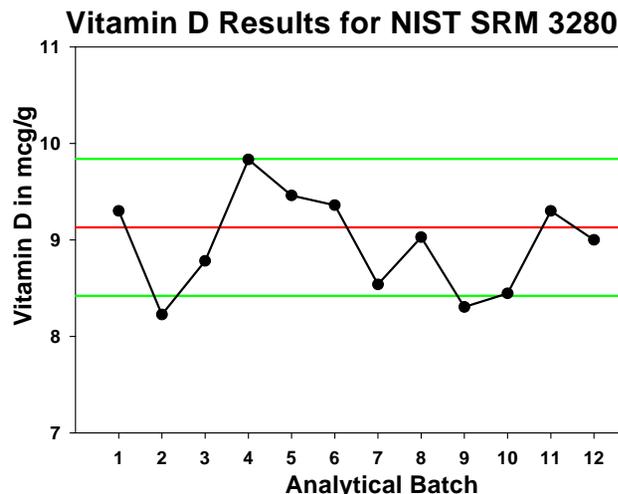


Figure 2

Vitamin D Results for NDL In-House Controls

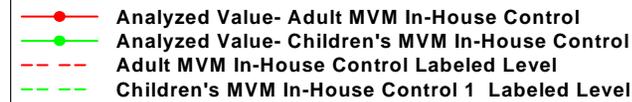
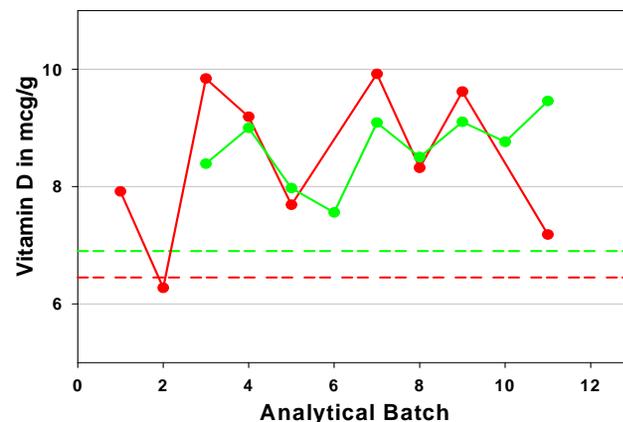


Table 1 – NDL Control Data

Control Sample	Lab Mean (µg/g)	Certified Value or Labeled Level (µg/g)	Percent Difference From Label or Certified Value	RSD	n
NIST SRM 3280	8.96	9.13	-2	6	12
Adult MVM	8.53	6.45	32	8	9
Children's Chewable MVM 1	8.48	6.90	23	14	10
Children's Chewable MVM 2	0.31	0.26	18	3	3

Results

Table 1 and Figure 1 show the NIST SRM 3280 results from this laboratory. Two of the results fell just below the NIST certified range, but the mean of 8.964 µg/g compared to 9.13 µg/g with a RSD of 6% was considered acceptable.

Along with the NIST SRM 3280, three MVM products were developed as in-house control materials. Table 1 and Figure 2 show the results of the in-house controls developed within these batches. The graphed in-house control materials have RSDs of 8% and 14%, respectively (an additional in-house children's chewable control material listed in Table 1 currently has an RSD of 3% (n=3)).

Blind product duplicates (2 sets of 20 tablets of the same MVM product) were also included in each of the analytical batches. Comparison of duplicate results (calculated by percent difference from the mean) ranged from 0-11% (n=17), 17-19% (n=2), and >35% (n=2).

No patterns or trends were obvious for the products for which duplicate results were significantly different from each other (e.g. product type, vitamin D form, market channel purchased). Inconsistent duplicate results may be due to product inhomogeneity and/or incomplete extraction due to differences in microencapsulation materials. However, the vast majority (>77%) of duplicate products displayed consistent results.

Conclusions

- The availability of NIST SRM 3280 has facilitated the development of an analytical method for vitamin D for the DSID study. Acceptable accuracy and precision for NIST SRM 3280 can be achieved with the method.
- Development of in-house controls allows monitoring of precision for the various matrices and ingredient levels available on the market.
- Most supplement products analyzed in duplicate using this method show consistent results (17 out of 21).

Future Plans

When the chemical analysis of vitamin D in adult and children's MVM products is complete, results will be analyzed statistically and reported in the DSID (www.dsid.usda.nih.gov).

References

- <http://its.nist.gov/measurementservices/referencematerials/index.cfm> - Accessed August 22, 2009
- <http://dietary-supplements.info.nih.gov/factsheets/vitaminD.asp> - Accessed August 31, 2009
- <http://www.dsid.usda.nih.gov>