Dietary Supplement Ingredient Database Project: Protocol Development

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Abstract

Scientific studies to assess the possible health benefits and risks of dietary supplement intake require accurate composition data on dietary supplements. A systematic approach to the chemical analysis of representative dietary supplement products is being implemented for the development of the Dietary Supplement Ingredient Database (DSID). Dietary supplement products were ranked based on the weighted frequency of their use (as reported in the National Health and Nutrition Evaluation Survey (NHANES) 1999-2000). Dietary supplement ingredients were ranked and seven high priority (Tier 1) nutrients were identified: folic acid, vitamin C, vitamin A (retinol, beta-carotene), vitamin E (alpha-tocopherol), calcium and iron. Multivitamins, the most frequently reported dietary supplement (30% of the reporting population taking supplements) were chosen for initial study. Standardized sample handling and analysis procedures were developed for the Tier 1 nutrients after reviewing results from several laboratories. These protocols, which address sample storage and homogenization, nutrient stability and encapsulation and analytical methods, will be used for the analysis of Tier 1 nutrients in representative multivitamin products. This project is funded by ODS/NIH Y4-HV-0051-05.

Objectives

- Identify and assess sample handling protocols and methods of analysis for multivitamin/mineral products.
- Assess the capabilities of analytical laboratories to determine the composition of vitamins and minerals in supplement products.
- Define appropriate sample handling and analysis protocols for seven nutrients in multivitamin/mineral matrices.

Introduction

The high prevalence of dietary supplement use in the US makes it vital to monitor the nutrients and other ingredients consumed in these products in order to obtain accurate intake information. As part of the Dietary Supplement Ingredient Database (DSID) working group, the Nutrient Data Laboratory at the United States Department of Agriculture is working with the Office of Dietary Supplements at the National Institutes of Health, the National Center for Health Statistics at the Center for Disease Control and the National Institute for Standards and Technology (NIST). This DSID working group is developing a database to provide representative values for the content of commonly used dietary supplement products.

In the first stage of this project, information on ingredient content and reported consumption patterns of dietary supplements was obtained from the NHANES 1999-2000 dietary supplement questionnaire (1). High-priority dietary supplement ingredients were identified using a series of weighted factors including exposure, research interest, measurement capabilities and public health importance. Seven Tier 1 nutrients (folic acid, vitamin C, vitamin A -- both retinol and beta-carotene, vitamin E, calcium and iron) were identified for initial pilot study work. These nutrients offer a range of processing challenges and nutrient types (2 minerals, 2 water soluble vitamins and 3 fat soluble vitamins).

Methods and Materials

Six laboratories were sent the following samples for the analysis of seven nutrients:

- 30 tablets Multivitamin /Multimineral Supplement (MVM 1)
- 30 tablets Multivitamin /Multimineral Supplement (MVM 2)
- Reference Materials (RMs)

  a. Milk-based product (RM 1) for retinol, vitamin C, folic acid, calcium, and iron
  b. Milk-based product (RM 2) for alpha-tocopherol
  c. Botanical matrix (RM 3) for beta-carotene
  d. Fortified grain product (RM 4), an additional retinol RM.

Each lab was sent two multivitamin tablets plus one or two reference materials. Four labs were selected to analyze these 3-4 samples for four nutrients based on laboratory experience, capabilities and to a lesser extent laboratory cost (Table 1). Labs were instructed to weigh and homogenize 20 tablets of multivitamin samples, or to mix the powder samples. Batch numbers were assigned to specific samples to insure that all samples for each nutrient were analyzed at the same time. Labs were given information about each sample matrix and approximate ranges expected for each nutrient. Blind duplicates were sent for selected samples and selected labs.

Table 1 Study Design: Analysis of Seven Nutrients in Six Samples by Six Laboratories

	Retinol	B-carotene	Vit C	A-tocopherol	Folic acid	Calcium	Iron
MVM1-Multivitamin	1,2,3,4 [a]	1,2,3,4	1,2,3,6	1,2,5,6	1,3,5,6	1,3,4,6	1,3,4,6
MVM2-Multivitamin	1,2,3,4	1,2,3,4	1,2,3,6	1,2,5,6	1,3,5,6	1,3,4,6	1,3,4,6
RM1-reference material 1	1,2,3,4		1,2,3,6		1,3,5,6	1,3,4,6	1,3,4,6
RM2-reference material 2	1,2,3,4						
RM3-reference material 3		1,2,3,4					
RM4-reference material 4				1,2,5,6			

[a] 1, 2, 3, 4, 5, 6 - each number represents one of the six laboratories chosen to analyze samples

Results

Sample Handling of Pills:

Four of the six laboratories weighed and homogenized 20 tablets of MVM sample. One of these four laboratories homogenized another 10 tablets to run a repeat analysis. A fifth laboratory used 12 tablets, and a sixth laboratory used 10 tablets to weigh and homogenize samples.

Homogenization equipment varied from lab to lab and included mortar and pestle, coffee mill or other high-speed mills. Time between homogenization and analysis ranged from 0-27 days. Storage temperature of the ground homogenate before analysis ranged from –30 to 30° C. Most laboratories used saponification in the analysis of the fat-soluble vitamins to break any potential encapsulation. However, one lab gave no mention of sample handling specific to encapsulation.

Methods of Analysis:

Three of the four laboratories used ICP to analyze samples for calcium and iron. One of these three labs used atomic absorption spectroscopy to analyze RM1for calcium and iron. The fourth has yet to disclose methods for calcium and iron.

For folic acid analysis, laboratories used both microbiological (2 labs) and HPLC (2 labs) methods. Analytical methods for the analysis of Vitamin C included both fluorometric (2 labs) and HPLC (2 labs). Retinol content was analyzed by HPLC for all four labs. Reverse-phase HPLC with spectrometry was used to quantify beta-carotene by 3 labs. The other lab used a spectrophotometric determination of beta-carotene. Alpha-tocopherol was quantified by HPLC for all four labs. Complete standard operating procedures for sample handling and nutrient analysis were difficult to obtain from several of the laboratories.

Figure 1

Alpha-tocopherol Values for 2 Multivitamin/mineral Products and a Reference Material

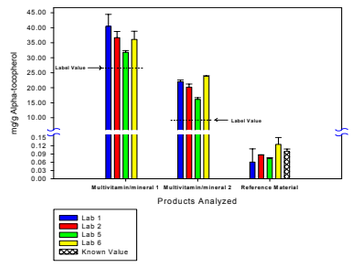


Figure 2

Iron Values for 2 Multivitamin/mineral Products and a Reference Material

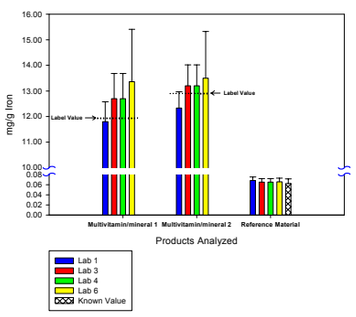
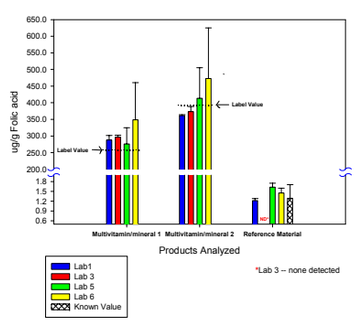


Figure 3

Folic Acid Values for 2 Multivitamin/mineral Products and a Reference Material



Results

Figures 1, 2 and 3 show means of analytical results for one fat-soluble vitamin (alpha-tocopherol), one mineral (iron), and one water-soluble vitamin (folic acid) respectively. For each of the three analyzed products in each graph, mean values are provided separately for each lab. In each graph, the reference material data is grouped last, with an extra bar representing the known value. Error bars for the known value represent acceptable limits for laboratory data, based on the quality of the RM data. Label values have been noted on the graphs as dotted lines. Label values are provided for informational purposes only since they may not indicate actual levels in the product. Additional data verification and statistical analysis are on going at this time.

Discussion

➢ Some of the analytical values for the three sample nutrients presented here are highly variable compared to the label claim. For others, the actual value is within 10% of the label claim. For alpha-tocopherol, the laboratories showed excellent consistency in their individual results, but all analytical values were significantly higher than the labeled value. For iron, consistency was seen in individual laboratory results and in agreement with label value for three of the four laboratories. For folic acid, only two laboratories showed consistency with individual results. Analytical folic acid values for the MVM products varied greatly from the label values. For these three nutrients, reference material values showed less variability than the values for the MVM products, with the exception of Laboratory 3 (folic acid below detection limit).

➢ The sample matrix and nutrient levels were vastly different in the reference materials than in the MVM samples. Currently available reference materials are food-based matrices and even fortified RMs have nutrient levels significantly lower than those in supplement products. Appropriate reference materials for the analysis of vitamins and minerals in dietary supplements are essential for validation of the analytical methods used to analyze these products and for quality control. Fortunately, a multiple vitamin/multiple element Standard Reference Material (SRM) is currently being developed by NIST.

➢MVM products are formulated for long term (over a year) storage at room temperature. Many manufacturers regularly add excess water-soluble and fat-soluble vitamins to products to allow for degradation throughout the shelf life. In addition, the more labile vitamins may be encapsulated with a fat-soluble coating to help reduce degradation. Sample preparation for these products must include an organic solvent to assure complete nutrient recovery. Optimum protocols still need to be defined in the following areas: size of homogenate, homogenization method, treatment of encapsulated nutrients, and storage time and temperature of homogenized sample. These variables all affect precision and accuracy of analysis.

Future Plans

1. An expert panel will be convened to review sample handling and analytical methodology specific to vitamin and mineral analysis in dietary supplement products. Recommended protocols will be used for future studies.
2. Statistical evaluation of the current data is not yet complete. Laboratories that are shown to have successfully analyzed for these nutrients will be candidates for the analysis of other vitamins and minerals in supplement products.
3. A pilot study designed to assess actual analytical variability between different products with the same label declaration for each of these Tier I nutrients will be initiated in the near future.

Reference

1. http://www.cdc.gov/nchs/data/nhanes/ Accessed May 2004