USE OF QUALITY CONTROL (QC) TO ASSESS THE VALIDITY OF RESULTS FOR ALPHA-TOCOPHEROL IN ADULT AND CHILDREN'S MULTIVITAMIN/MINERAL (MVM) SUPPLEMENTS

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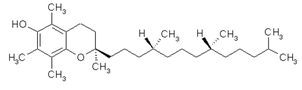
ABSTRACT

Vitamin E is a fat-soluble antioxidant that exists in eight chemical forms of tocopherols and tocotrienols. Out of the group of eight, the most biologically active form of vitamin E is alpha-tocopherol. Many multivitamin/mineral (MVM) products sold in the U.S contain vitamin E and alpha-tocopherol is most commonly added to those supplements in one of two forms, the natural (RRR-alpha-tocopherol) or the synthetic (all-rac-alpha-tocopherol).The distinction of vitamin E form in dietary supplements is important since the labeled level is reported in International Units (IU) and the conversion factor for each form differs. The most commonly labeled level is 30 IU, which is 100% of the Daily Value (DV). The alpha-tocopherol content of MVMs has been chemically evaluated as part of the Dietary Supplement Ingredient Database (DSID) project at the Nutrient Data Laboratory (NDL) and the analytical values have been compared to labeled levels. An experienced commercial laboratory with validated analytical methods was chosen to perform the analyses using HPLC with fluorescence detection. In order to evaluate the precision and accuracy of the alpha-tocopherol analyses, a number of QC materials were added to each batch of samples sent to the lab. In each set of 20 samples a National Institute of Standards and Technology Standard Reference Material (NIST SRM) 3280, a multivitamin/mineral with certified levels of alpha-tocopherol was included. Additional in-house control materials, 2-3 samples were added as well. For alpha-tocopherol in the adult MVM study, the laboratory mean for NIST SRM 3280 was -3.4% (n=20) of the certified value with a relative standard deviation (RSD) of 6.4% (certified range +/-16.6%). The two in-house control materials had RSDs of 9.1% (n=19) and 8.2% (n=29). For children's MVMs, the laboratory mean for NIST SRM3280 was +3.0% (n= 14) of the certified value with a RSD of 4.7% while the two in-house control materials showed RSDs of 5.1 and 6.9% (n=17, 23). These control material results are used to assess and validate product results for DSID studies. National mean estimates reported for vitamin E in MVM products in the DSID (http://dietarysupplementdatabase.usda.nih.gov) predict a +6.0% difference from label for adult MVMs and a +14.0% difference from label in children's MVMs at their most commonly labeled levels. In March 2012, NDL released DSID-2 which reports ingredient levels for vitamins and minerals in MVMs.

INTRODUCTION

RRR-alpha-tocopherol



Vitamin E is an ingredient of interest as it is widely known for its health benefits and protection against toxins. The antioxidant properties of Vitamin E remove free radicals and strengthen the immune system. It is a fat-soluble antioxidant, available in many foods and dietary supplements[1]. The DSID project is a collaborative project that is funded by the NIH Office of Dietary Supplements and collaboratively administered with the U.S. Department of Agriculture. The objective is to chemically evaluate the levels of ingredients in dietary supplement products and publish national estimates for researchers studying the health impacts of food and supplement intake. For every study, a sampling plan is established to identify and select representative dietary supplements that, when analyzed, can provide reliable and representative estimates of means and variability for nutrient content. In the adult MVMs study, 86 out of 115 products contained Vitamin E. Of these, 28 products contained the natural form of Vitamin E (RRR-alpha-tocopherol) while 58 had the synthetic form (all-rac-alpha-tocopherol). For the children's MVMs study, 62 out of 65 products analyzed contained Vitamin E and the breakdown for synthetic or natural Vitamin E is 44 and 18, respectively. The natural source of Vitamin E comes from vegetable oils while the synthetic form is a mixture of eight alpha-tocopherol stereoisomers in equal amounts. In order to monitor the accuracy and precision of the alpha-tocopherol results for DSID studies, a quality assurance and control plan was developed and implemented.

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METHOD OF ANALYSIS AND QUALITY CONTROL

For both the adult and children's MVM studies, QC materials were included in every batch sent out for analysis. Each batch contained 18-20 different samples and each sample contained 20 units (tablets, capsules) of the product. The following is included in a typical batch:

- A sample (20 tablets) of NIST SRM3280
- A set of product duplicates (two sets of 20 units of the same MVM product with different test sample IDs)
- A sample (20 tablets) of two different in-house control materials
- Product samples (15). For tablets and capsules, 20 units were sent. For powder and liquid samples, a comparable amount of material was weighed out and sent for analysis to our contracted laboratories.

Each sample was homogenized, treated with enzyme, and saponified with KOH to release the tocopherols. The sample was then extracted with organic solvent, followed by separation and quantification on an HPLC silica column using fluorescence detection.

QC RESULTS:

Figure 1

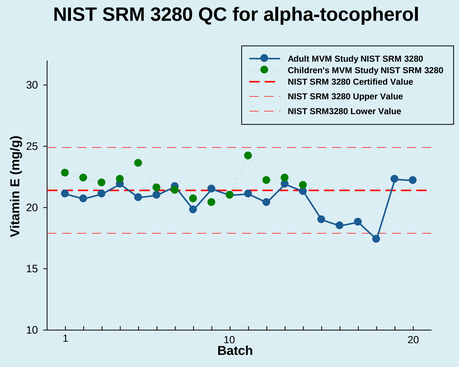

Figure 2

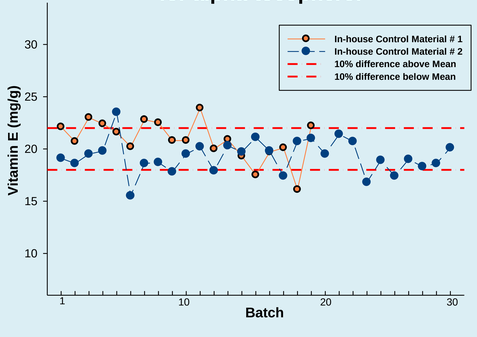

Figure 3

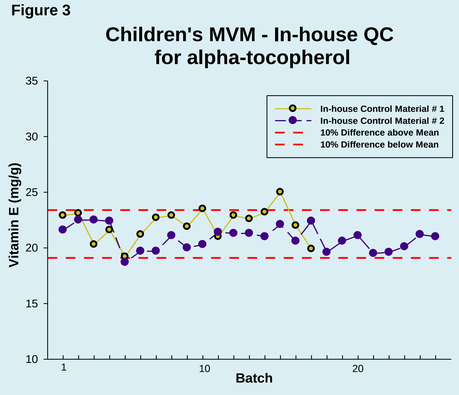

Table 1: Example of Batch QC Summary table

MVMs Study	Batch ID	Ingredient	% Difference from NIST Certified Value	% Difference from Mean of Control 1	% Difference from Mean of Control 2	QC Summary
Adult MVMs	# 111	Alpha-tocopherol	-2	+1	-1	Good
	#112	Alpha-tocopherol	-11	-1	-7	Low bias
Children's MVMs	# 010	Alpha-tocopherol	+3	+4	0	Good
	# 052	Alpha-tocopherol	-9	0	+8	Variable

RESULTS AND DISCUSSION

Quality control data indicated good quality results for dietary supplement products analyzed for Vitamin E (alpha-tocopherol) content for the DSID study. Figure 1 shows the analytical results for alpha-tocopherol in NIST SRM 3280, which was sent in all adult MVM and children's MVM batches. The certified value for alpha-tocopherol is 21.4 mg (+/- 3.5) and these upper and lower ranges are identified on the graph. The results for all but one batch fell within this range. The NIST mean result for the adult MVM study is -3.4% of the certified value with an RSD of 6.4%. The NIST mean result for the children's study is +3.0% of the certified value with an RSD of 4.7%. Figures 2 & 3 show the quality control results for in-house control materials, with reference lines indicating +/-10% of the mean value for the adult and children's MVM study respectively. In the adult study, there are 6 batches with results higher than +/- 10% of the mean while the children's study only had one batch out of this range.

Table 1 shows an example of the QC summary charts developed for each study, with calculated percentage differences from certified and mean values for SRM and in-house control materials. This QC chart is used to evaluate the quality of the product data received in each batch. The batch QC was identified as good, variable, questionable with either a low or high bias, or unacceptable. Product results for batches with QC results beyond the +/-10% range for in-house materials and outside of the certified range for NIST SRM3280, were evaluated more critically and retested if questionable. For batches with unacceptable QC data, discussion with the laboratory may be necessary to solve the problem. However, none were evaluated as unacceptable.

Duplicate results are also assessed for each batch. Extremely high or low percentage difference from mean can indicate a problem with the product results in the batch. Product results showing unusually high or low results compared to labeled levels or high variability among product lots were identified for retesting, even if QC data for these batches was acceptable. After retesting, analytical results for each sample were finalized for each study. Laboratory results reported in mg/g and were converted to IUs based on the form of the vitamin E. These values were compared to labeled levels and a percent difference from label was calculated. Statistical evaluation of the data determined national estimates based on regression predictions.

DSID
Dietary Supplement Ingredient Database
A U.S. Government Initiative

NEXT STEPS

1. These QC protocols will continue to be used to assess the quality of the analytical results obtained for ingredients in dietary supplements. Current DSID studies include an omega-3 (n-3) fatty acids supplement study and a prenatal MVM study. In addition we have begun a second study of adult MVMs which will monitor changes over time for some ingredients and evaluate additional ingredients of interest (iodine, vitamins A and D, chromium) which have not been published in previous DSID releases.

2. A study of the ingredient content in botanical supplements is in the planning process. QC protocols may need to be expanded for botanicals due to the need for identity testing, in addition to the accurate quantification of ingredients.

3. The next release of the DSID (DSID-3) data is planned for 2014. This release will report on the vitamin and mineral content of over-the-counter prenatal MVM products and the EPA, DHA and ALA content of omega-3 fatty acid supplements.

Reference:

[1]Evert, A. (February 8, 2011). Vitamin E Fact Sheet. In *Medline Plus: Trusted Health Information for You*. Retrieved April 12, 2013, from http://www.nlm.nih.gov/medlineplus/ency/article/002406.htm