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Original Article

Development and validation of control materials for the measurement of vitamin D_3 in selected US foods

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ABSTRACT

As part of the United States Department of Agriculture's (USDA) National Food and Nutrient Analysis Program (NFNAP), food composition data for vitamin D in the USDA National Nutrient Database for Standard Reference are being updated and expanded, focusing on high priority foods and validated analytical methodology. A lack of certified reference materials and analytical methods validated for these key foods required the development of five matrix-specific control composite materials (CC) (canned salmon and vitamin D_3 fortified cereal, orange juice, milk, and cheese). Each of six experienced laboratories (research and commercial) analyzed vitamin D_3 in five subsamples of each CC in five separate analytical batches, with one subsample of each material in each run. Research laboratories performed recovery studies, mass spectrometric analysis, and other studies to validate quantitation in each matrix. Initial results showed a wide disparity between the six laboratories (RSDs of 26–46%). Extensive collaboration resolved several problems related to calibration standards, extraction solvents and the internal standard, achieving final values with RSDs of approximately 10%, validated by mass spectrometry tests that confirmed lack of matrix interferences in these foods.

1. Introduction

There has been increased interest recently by the scientific community in the role of vitamin D in health beyond preventing rickets or osteomalacia. Evidence suggests that raising levels of the circulating form of vitamin D in serum may result in improved bone health, oral health, and colon cancer prevention (Bischoff-Ferrari et al., 2006). While sunlight induces cutaneous vitamin D synthesis thus increasing serum levels, there are multiple factors that can reduce an individual's exposure to sunlight (Holick, 2007; Calvo et al., 2004), making reliance on foods or supplements with added vitamin D more important (Chen et al., 2007). Vitamin D occurs in foods naturally primarily as vitamin D_3 (cholecalciferol) (Holick, 2007) and also as vitamin D_2 (ergocalciferol) in plants and 25-hydroxyvitamin D_3 in animal products such as meat and eggs (Ovesen et al., 2003; Mattila et al., 1996). In the USA, vitamin D_3 is used as a fortificant for most foods (e.g., milk, orange juice, cheese, cereals), although vitamin D_2 is sometimes used (primarily in soy and vegetarian products).

Many epidemiological studies of vitamin D are based on calculated intake using consumption data from dietary surveys and vitamin D concentrations from food composition databases (Affenito et al., 2007; Gilmore et al., 2008). Population estimates of dietary intakes in the US are estimated through the dietary component of the National Health and Nutrition Examination Survey (NHANES) using the Food and Nutrient Database for

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Dietary Surveys (FNDDS) (US Department of Agriculture, 2006). The source of nutrient data for the FNDDS is the United States Department of Agriculture's (USDA) National Nutrient Database for Standard Reference (SR) (US Department of Agriculture, 2007), which is maintained by the Nutrient Data Laboratory (NDL) at the Beltsville Human Nutrition Research Center, an institute of the Agricultural Research Service (Beltsville, MD). The SR contains data for 7500 foods, approximately 2700 of which are used in the FNDDS. Current epidemiological work on vitamin D intake, whether through NHANES or through other surveys, is impaired by the lack of a complete, and well verified, database of vitamin D values for foods commonly eaten in the US. SR currently contains vitamin D values for only 594 foods and just 87 of those are analytical values. Thus far, data on vitamin D has been presented in SR as total vitamin D in IU/100 g because IU is the unit required under the current US labeling regulations, and most of the data on vitamin D to date have been provided by industry or based on US standards of identity. Beginning in 2009, vitamin D data will be reported in SR as micrograms D_2 or D_3, and total vitamin D in micrograms will be calculated from the sum of specific forms, with eventual provision of values for 25-hydroxyvitamin D for some foods. Currently, 25-hydroxy vitamin D values (Ovesen et al., 2003) are included in the UK food tables for meats (Chan et al., 1995).

To support these research needs, NDL has initiated an update and expansion of food composition data for vitamin D in SR (Holden et al., 2008), using accurate, validated sampling and analytical methodology. This work is being conducted as part of NDL's National Food and Nutrient Analysis Program (NFNAP) (Pehrsson et al., 2000; Haytowitz et al., 2002, 2008). The foods likely to supply the most vitamin D in the US diet were identified as fish (a natural contributor) and the following vitamin D_3 fortified foods: orange juice, ready-to-eat breakfast cereals, fluid milk, margarines, sliced American cheese, and yogurt; therefore, these foods were given the highest priority for new chemical analyses, focusing on vitamin D_3 (Holden et al., 2008).

The NFNAP protocol requires that analytical methods used are verified for accuracy and acceptable precision and that control samples and/or certified reference materials (CRMs) are included in all assay batches to provide validation of individual datasets and continuity of results across time, laboratories, and methodology (Phillips et al., 2006). Usually CRMs with a known nutrient content can be included to evaluate the accuracy of results from prospective analytical laboratories. In the case of vitamin D, however, there is a lack of CRMs for the primary food sources (Phillips et al., 2007), which presents a major obstacle to validating methodology and laboratories for the foods planned for analysis. Existing CRMs include only infant formula (NIST SRM 1846; National Institute of Standards and Technology (NIST), Gaithersburg, MD), margarine (BCR122; Institute for Reference Materials and Methods (IRMM), Geel, Belgium), and powdered milk (BCR 421). The vitamin D value for the NIST Infant Formula is a reference value, not a certified concentration, indicating less confidence. BCR 421 has a certified value, but the material is over 10 years old and has recently been discontinued. Dry matrices (e.g., powdered milk and infant formula) may not be analytically equivalent to their fluid counterparts, because water content and nutrient concentration can affect selection and performance of the extraction, separation, and quantitation. Margarine (BCR 122) is dissimilar to the primary food matrices to be assayed in NFNAP. The peanut oil matrix and vitamin D_3 concentration (80,000 IU/100 g) in the commercially available USP standard are not equivalent to the foods to be assayed. Therefore, a set of matrix-matched control materials was needed for the NFNAP.

Additionally, preliminary data obtained during prior NFNAP phases for available CRMs analyzed along with a limited number of food samples submitted to major commercial laboratories raised uncertainty about the adequacy of precision and accuracy of measurements. The CRM results showed high variability and often deviated significantly from the certificate values; also, replicate analyses of vitamin D in control materials prepared for the NFNAP study (Phillips et al., 2006) showed poor repeatability and obvious errors in many cases (e.g., a high value in a mixed vegetable control material) (Holden et al., 2008; Holden et al., unpublished data). These observations generated fundamental uncertainty about the reliability of existing standard methods for the range of food matrices representing primary sources of vitamin D in the US and motivated studies to validate measurements using the control materials prior to the planned analysis of foods for NFNAP.

Historically, the determination of vitamin D in foods has presented an enormous analytical challenge because the chemistry of this vitamin is complex and the methods are detailed and time consuming. There are 11 methods validated by AOAC International (Gaithersburg, MD), the US organization responsible for establishing official methods that are legally defensible. Three chemical methods have been published since 1990: Method 992.26—vitamin D_3 (cholecalciferol) in ready-to-feed milk-based infant formula (AOAC, 2007b), Method 995.05—vitamin D in infant formulas and enteral products (AOAC, 2007a), and Method 2002.05—cholecalciferol (vitamin D_3) in selected foods (milk and cheese) (AOAC, 2007d). One older method, Method 982.29—vitamin D in mixed feeds, premixes, and pet foods also has applicability (AOAC, 2007c).

All the methods are quite similar. In general, samples are saponified to hydrolyze the lipids, vitamin D_2 and D_3 are extracted, then both vitamin D_2 and D_3 are collected as a single peak using preparative scale normal-phase high performance liquid chromatography (HPLC) and vitamin D_2 and D_3 are separated using analytical reversed-phase chromatography with diode array detection. Variations arise from the different extraction solvents used (usually either hexane or ether/petroleum ether) and the use of internal standards (IS).

There are two major problems with existing standard methods for fortified foods. First, they are long, labor-intensive, and require extreme attention to detail. This creates the potential for error and poor precision, resulting in a tendency to run as few samples as necessary, and also requiring a skilled analyst. Consequently, the relative standard deviation (RSD) of data generated by these methods tends to be high and is at odds with the inclination to run fewer samples. More, not fewer, samples are needed to achieve assayed concentrations with an acceptable level of confidence when there is relatively low precision. Second, the methods were only validated for vitamin D_3 fortified dairy products, and not other types of food. Expansion of the applicability of the methods is mandatory for reliable overall food composition data. This is especially critical since many of the newest fortified foods (e.g., orange juice and cereals) have different matrix characteristics that might affect extraction and separation of vitamin D_3. Also, naturally occurring vitamin D (e.g., in meat, fish, eggs) may not be extracted as efficiently as it is from fortified foods, as well as also being present as 25-hydroxy vitamin D (Jakobsen et al., 2004; Mattila et al., 1996) or, in foods such as mushrooms, as vitamin D_2 (ergocalciferol) (Mattila et al., 2002).

The primary goal of this study was to prepare and characterize control materials for vitamin D_3 in specific food matrices to enhance the quality of analytical measurements of vitamin D in key foods for the NFNAP, to ensure the accuracy and consistency of new SR data, and to harmonize results from methods among a subset of laboratories.