ANALYSIS OF VITAMIN D AND PREVITAMIN D IN FOODS
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INTRODUCTION

The U.S. Food and Drug Administration (FDA) revised the food labeling regulations in 2016, to make the vitamin D content a required item on the nutrition or supplement facts labels for conventional food and dietary supplements. The change in labeling regulations is aimed to promote vitamin D awareness among consumers. Recently, a new AOAC standard method which uses a derivatization reaction (PTAD) in the sample prep and determination of vitamin D by mass spectrometry is available. This new method has provided much better analytical performance for vitamin D analysis than previous methods. However, previtamin D is not measured in this new standard method. Precursors of vitamin D are bioactive, and it is known that Vitamin D can thermally isomerize to previtamin D (Figure 1). It has been reported that the relative content of previtamin D could be up to 22% of the total vitamin D content. Therefore, it is prudent to count previtamin D content in the analysis of vitamin D in foods.

This work proposes a new method in the determination of total vitamin D by individually measuring the vitamin D and previtamin D in food products.

METHODS

Sample Preparation:

Previtamin D and vitamin D concentrations of the food samples were determined using the method described in AOAC INTERNATIONAL (2016). The food samples were blended with acetonitrile/methanol and then centrifuged. The supernatant was filtered and injected into the UPLC system. The vitamin D and previtamin D were separated on a Waters ACQUITY UPLC BEH C18 column (2.1 × 100 mm, 1.7 μm). The mobile phases were A) of 0.1% Formic acid in water and B) of acetonitrile. The total run time was 6.5 minutes. The data were processed using Waters MassLynx V4.1 software. Relative response factors were determined each time the samples were analyzed. The results of vitamin D analysis for the NIST reference sample showed excellent accuracy (102.6%), and repeatability (2.4%).

RESULTS

Typical UPLC chromatograms of vitamin D and previtamin D in standard mix solutions and infant formula samples are separated from previtamin Ds (3.50 min) to vitamin Ds (3.67 min).

Calibration plots of vitamin D

The calibration process is illustrated in Figure 3. The total vitamin D peak area was calculated according to the following equation:

\[ \text{Total VitD Peak Area} = \text{Total VitD Peak Area}_{\text{previtamin D}} + \text{Total VitD Peak Area}_{\text{vitamin D}} \]

Relative response factors for vitamin D3, vitamin D2, and SIL-D3 were estimated at 0.01 mg/kg and 0.02 mg/kg in oatmeal, and 0.0003 mg/kg and 0.002 mg/kg in solvent, respectively. The LOQ values are comparable among consumers.

Method performance and analysis results

Table 4 shows the LOQs in oatmeal were 0.01 mg/kg and 0.0003 mg/kg (D3) and 0.002 mg/kg (D2) for vitamin D3 and vitamin D2, respectively. These samples are comparable to the existing standard methods. Good agreement with the label values have been observed for the infant formula, dry milk powder, and oatmeal. A variety of food products, such as non-fat dry milk powder, infant formula (sex based), chocolate, oatmeal, and fish oil samples have been successfully tested with this method. Good agreement of the label values have been observed for the infant formula, dry milk powder, and oatmeal.

Benefits of measuring previtamin D in the vitamin D analysis

Vitamin D analysis methods have the ability to consider previtamin D in total vitamin D levels. This method is able to provide a complete vitamin D analysis and can avoid misinterpreting the vitamin D intake of the population using the label value as the only source. The analysis systems are simple and require less time for analysis. The results of this method are more accurate and can avoid the misinterpreting of vitamin D intake. The new method has been successfully tested with this method. Good agreement of the label values have been observed for the infant formula, dry milk powder, and oatmeal.

REFERENCES

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DISCUSSION

Vitamin D and previtamin D in food products are separated from previtamin Ds (3.50 min) to vitamin Ds (3.67 min).

Figure 2. Typical UPLC chromatograms of vitamin D and previtamin D in food products.