

## Standard Method Performance Requirements (SMPRs) for Determination of Vitamin D in Dietary Supplement Finished Products and Ingredients

### 1 Applicability

The method will separate and accurately quantitate vitamin D<sub>2</sub> (ergocalciferol), vitamin D<sub>3</sub> (cholecalciferol), and their previtamin D forms, and, if possible, the 25-hydroxy forms in dietary supplement finished products and the ingredients used to formulate these products. See Figure 1.

### 2 Analytical Technique

Any analytical technique that meets the following method performance requirements is acceptable.

### 3 Definitions

**Dietary ingredients.**—Vitamin; mineral; herb or other botanical; amino acid; dietary substance for use by man to supplement the diet by increasing total dietary intake; or a concentrate, metabolite, constituent, extract, or combination of any of the above dietary ingredients {United States Federal Food Drug and Cosmetic Act §201(ff) [U.S.C. 321 (ff)]}.

**Dietary supplements.**—Product intended for ingestion that contains a “dietary ingredient” intended to add further nutritional value to (supplement) the diet. Dietary supplements may be found in many forms such as tablets, capsules, softgels, gelcaps, liquids, or powders.

**Limit of quantitation (LOQ).**—Minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.

**Repeatability.**—Variation arising when all efforts are made to keep conditions constant by using the same instrument and operator and repeating during a short time period. Expressed as the repeatability standard deviation (SD<sub>r</sub>); or % repeatability relative standard deviation (%RSD<sub>r</sub>).

**Reproducibility.**—Standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility standard deviation (SD<sub>R</sub>); or % reproducibility relative standard deviation (%RSD<sub>R</sub>).

**Recovery.**—Fraction or percentage of spiked analyte that is recovered when the test sample is analyzed using the entire method.

### 4 Method Performance Requirements

See Tables 1 and 2.

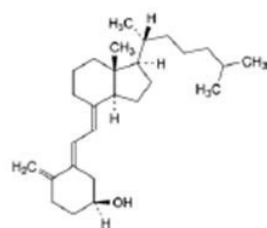
### 5 System Suitability Tests and/or Analytical Quality Control

Suitable methods will include blank check samples, and check standards at the lowest point and midrange point of the analytical range. A control sample must be included.

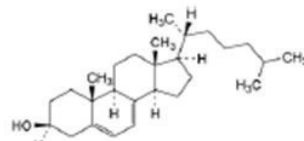
### 6 Reference Material(s)

NIST Standard Reference Material® 3280; the reference value of vitamin D<sub>2</sub> in NIST 3280 is 8.6 µg/g (±2.6) µg/g vitamin D<sub>2</sub>.

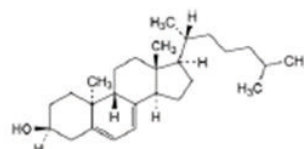
NIST Standard Reference Material® 3532; the reference value of vitamin D<sub>3</sub> in NIST 3532 is 1.310 ± 0.033 µg/g cholecalciferol (vitamin D<sub>3</sub>).



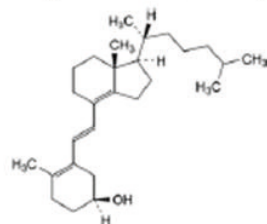
A. (5E,7E)-9,10-secocholesta-5,7,10(19)-trien-3β-ol (trans-cholecalciferol, trans-vitamin D<sub>3</sub>).



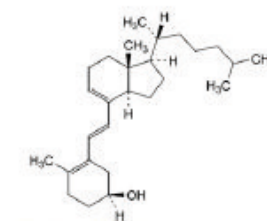
B. cholesta-5,7-dien-3β-ol (7,8-didehydrocholesterol, provitamin D<sub>3</sub>).



C. 9β,10α-cholesta-5,7-dien-3β-ol (lumisterol<sub>3</sub>).



D. (6E)-9,10-secocholesta-5(10),6,8(14)-trien-3β-ol (iso-tachysterol<sub>3</sub>).



E. (6E)-9,10-secocholesta-5(10),6,8-trien-3β-ol (tachysterol<sub>3</sub>).

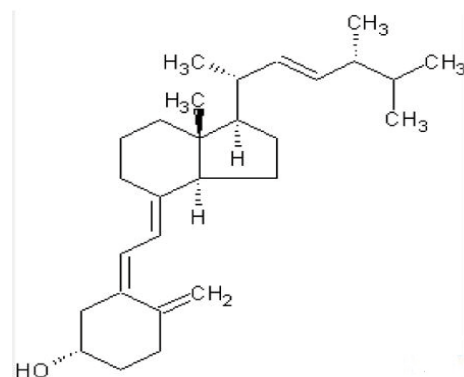


Figure 1. Chemical structure of vitamin D<sub>2</sub> (ergocalciferol), vitamin D<sub>3</sub> (cholecalciferol), and their previtamin D and hydroxy forms.

Parameter	Finished products	Ingredients
Analytical range ppm <sup>a</sup>	0.5–12 500	1250–12 500
Limit of quantitation ppm <sup>a</sup>	≤0.4	1000

<sup>a</sup> Measured as individual forms of vitamin D and pre-vitamin D.

## 7 Validation Guidance

*Appendix D: Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis, Official Methods of Analysis (20th Ed.), AOAC INTERNATIONAL, Rockville, MD, USA ([http://www.eoma.aocac.org/app\\_d.pdf](http://www.eoma.aocac.org/app_d.pdf))*

*Appendix K: Guidelines for Dietary Supplements and Botanicals, Official Methods of Analysis (20th Ed.), AOAC INTERNATIONAL, Rockville, MD, USA ([http://www.eoma.aocac.org/app\\_k.pdf](http://www.eoma.aocac.org/app_k.pdf)). Also at: *J. AOAC Int.* **95**, 268(2012); DOI: 10.5740/jaoacint.11-447*

## 8 Maximum Time-to-Determination

No maximum time.

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*Approved by the AOAC Stakeholder Panel on Dietary Supplements (SPDS). Final Version Date: September 25, 2015. Effective Date: September 25, 2015. Revised March 2017.*

Parameter	Range, µg/g <sup>a</sup>				
	<10–15	>15–50	>50–500	>500–4000	>4000–12 500
Recovery, %	80–110	90–107	95–105	95–105	97–103
Repeatability (RSD <sub>r</sub> ), %	≤8	≤7	≤5	≤4	≤3
Reproducibility (RSD <sub>R</sub> ), %	≤12	≤10	≤8	≤6	≤4

<sup>a</sup> Measured as individual forms of vitamin D and pre-vitamin D.