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

1 Applicability

The method will separate and accurately quantitate vitamin D_2 (ergocalciferol), vitamin D_3 (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 De initions

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_r); or % repeatability relative standard deviation (%RSD_r).

Reproducibility.—Standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility standard deviation (SD_R); or % reproducibility relative standard deviation (% RSD_R).

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.

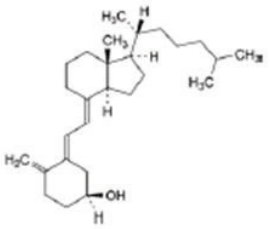
5 System Suitability Tests and/or Analytical Quality Control

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.

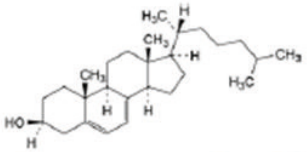
6 Reference Material(s)

NIST Standard Reference Material® 3280; the reference value of vitamin D_2 in NIST 3280 is 8.6 µg/g (±2.6) µg/g vitamin D_2.

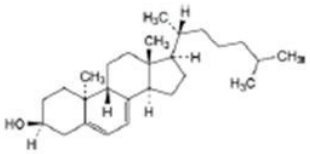
NIST Standard Reference Material® 3532 D_3; the reference value of vitamin D_3 in NIST 3532 is 1.310 ± 0.033 µg/g cholecalciferol (vitamin D_3).



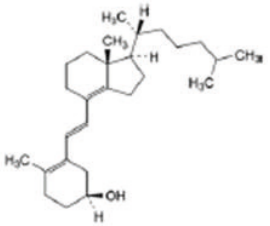
A. (5*E*,7*E*)-9,10-secocholesta-5,7,10(19)-trien-3β-ol (*trans*-cholecalciferol, *trans*-vitamin D_3),



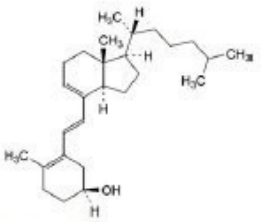
B. cholesta-5,7-dien-3β-ol (7,8-didehydrocholesterol, provitamin D_3),



C. 9β,10α-cholesta-5,7-dien-3β-ol (lumisterol$_3$),



D. (6*E*)-9,10-secocholesta-5(10),6,8(14)-trien-3β-ol (iso-tachysterol$_3$),



E. (6*E*)-9,10-secocholesta-5(10),6,8-trien-3β-ol (tachysterol$_3$).

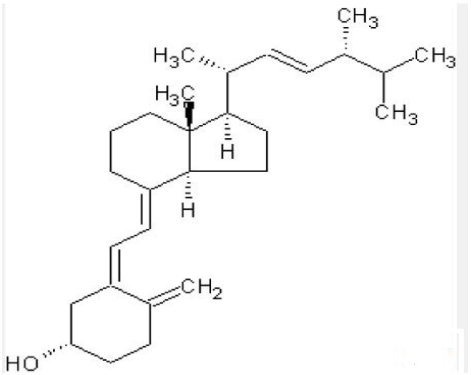


Figure 1. Chemical structure of vitamin D_2 (ergocalciferol), vitamin D_3 (cholecalciferol), and their previtamin D and hydroxy forms.

7 Validation Guidance

Appendix D: Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis, *Official Methods of Analysis* (current edition), AOAC INTERNATIONAL, Rockville, MD, USA. Available at: http://www.eoma.aoac.org/app_d.pdf

Appendix K: Guidelines for Dietary Supplements and Botanicals, *Official Methods of Analysis* (current edition), AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.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.

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

Table 1. Analytical range and LOQ based on matrix

Parameter	Finished products	Ingredients
Analytical range ppm[a]	0.5–12 500	1250–12 500
Limit of quantitation ppm[a]	0.4	1000

[a] Measured as individual forms of vitamin D and pre-vitamin D.

Table 2. Method performance requirements as a function of range

	Range, µg/g[a]				
Parameter	<10–15	>15–50	>50–500	>500–4000	>4000–12 500
Recovery, %	80–110	90–107	95–105	95–105	97–103
Repeatability (RSD_r), %	8	7	5	4	3
Reproducibility (RSD_R), %	12	10	8	6	4

[a] Measured as individual forms of vitamin D and pre-vitamin D.