DRAFT AOAC SMPR 2015.XXX; Version 4; June 12, 2015

Method Name: **Determination of Vitamin D in Dietary Supplement Consumer Products and Raw Materials**

Approved by: Stakeholder Panel on Dietary Supplements

Intended Use:

1. **Applicability**:
   The method will separate and accurately quantitate vitamin D_2 (ergocalciferol), vitamin D_3 (cholecalciferol), and their previtamin d and hydroxy forms in dietary supplement consumer products and the raw materials used to formulate these products.

2. **Analytical Technique**:
   Any analytical technique that meets the following method performance requirements is acceptable.

3. **Definitions**:

   Dietary ingredients.— A vitamin; a mineral; an herb or other botanical; an amino acid; a 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.— A 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) .— The 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.— The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility relative standard deviation (SD_R); or % reproducibility relative standard deviation (% RSD_R).

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

4. **Method Performance Requirements**:

Table 1: Analytical Range & LOQ Based on Matrix

Parameter	Finished Products	Raw Materials
Analytical range ppm*	0.5 – 338	1,250 - 12,500
Limit of Quantitation ppm*	0.4	1,000

Table 2: Method Performance Requirements as a Function of Range

Parameter	Ranges (µg/g)*				
	< 10 - 15	>15 - 50	>50 – 500	>500 – 4,000	>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

* Measured as individual forms of Vitamin D and pre-Vitamin D

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 micrograms/gram (±2.6) mg/kg of vitamin D_2.

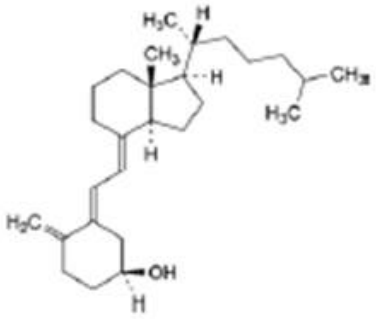
7. **Validation Guidance**:
Appendix D: Guidelines for Collaborative Study Procedures To Validate Characteristics of a Method of Analysis; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_d.pdf

Appendix K: Guidelines for Dietary Supplements and Botanicals 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Also at: . AOAC Int. 95, 268(2012); DOI: 10.5740/jaoacint.11-447 and available at: http://www.eoma.aoac.org/app_k.pdf

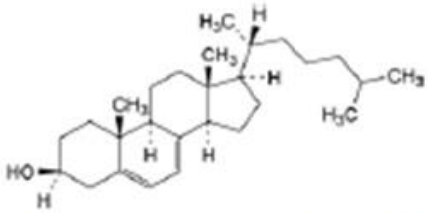
8. **Maximum Time-To-Determination:** No maximum time.

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

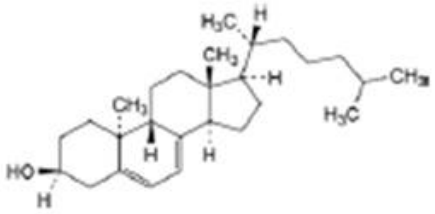
85



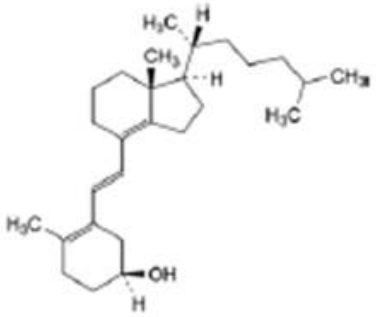
A. (5*E*,7*E*)-9,10-secocholesta-5,7,10(19)-trien-3β-ol (*trans*-cholecalciferol, *trans*-vitamin D_3),



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

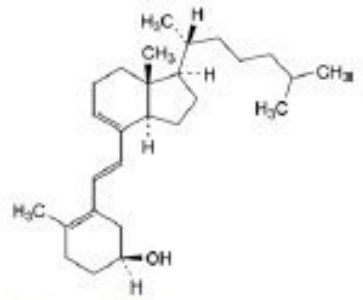


C. 9β,10α-cholesta-5,7-dien-3β-ol (lumisterol$_3$),



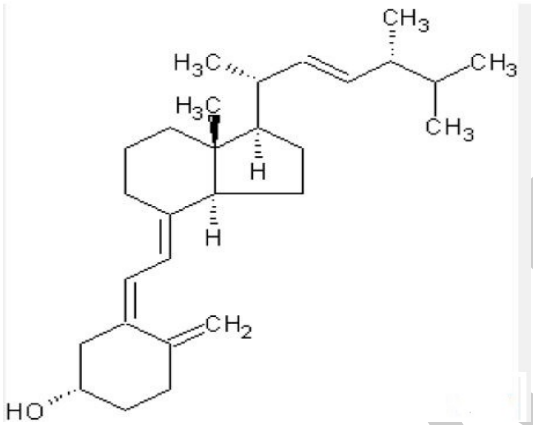
D. (6*E*)-9,10-secocholesta-5(10),6,8(14)-trien-3β-ol (iso-tachysterol$_3$),

86



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

87



88