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 D₂ (ergocalciferol), vitamin D₃ (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_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 D₂ in NIST 3280 is 8.6 μg/g (±2.6) μg/g vitamin D₂.

NIST Standard Reference Material® 3532; the reference value of vitamin D₃ in NIST 3532 is 1.310 ± 0.033 μg/g cholecalciferol (vitamin D₃).

Figure 1. Chemical structure of vitamin D₂ (ergocalciferol), vitamin D₃ (cholecalciferol), and their previtamin D and hydroxy forms.
7 Validation Guidance


8 Maximum Time-to-Determination

No maximum time.


Table 1. Analytical range and LOQ based on matrix

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Finished products</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical range ppm(^a)</td>
<td>0.5–12 500</td>
<td>1250–12 500</td>
</tr>
<tr>
<td>Limit of quantitation ppm(^a)</td>
<td>≤0.4</td>
<td>1000</td>
</tr>
</tbody>
</table>

\(^a\) Measured as individual forms of vitamin D and pre-vitamin D.

Table 2. Method performance requirements as a function of range

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range, (\mu g/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;10–15</td>
</tr>
<tr>
<td>Recovery, %</td>
<td>80–110</td>
</tr>
<tr>
<td>Repeatability (RSD(_r)), %</td>
<td>≤8</td>
</tr>
<tr>
<td>Reproducibility (RSD(_R)), %</td>
<td>≤12</td>
</tr>
</tbody>
</table>

\(^a\) Measured as individual forms of vitamin D and pre-vitamin D.