AOAC SMPR® 2016.017

Standard Method Performance Requirements (SMPRs) for Quantitative Measurement of Vitamin B_{12} in Dietary Supplements and Ingredients

Intended Use: Reference Method for cGMP Compliance

1 Purpose

AOAC SMPRs describe the minimum recommended performance characteristics to be used during the evaluation of a method. The evaluation may be an on-site verification, a single-laboratory validation, or a multi-site collaborative study. SMPRs are written and adopted by AOAC stakeholder panels composed of representatives from industry, regulatory organizations, contract laboratories, test kit manufacturers, and academic institutions. AOAC SMPRs are used by AOAC expert review panels in their evaluation of validation study data for methods being considered for *Performance Tested Methods*^{SM} or AOAC *Official Methods of Analysis*^{SM}, and can be used as acceptance criteria for verification at user laboratories. [Refer to Appendix F: *Guidelines for Standard Method Performance Requirements*, *Official Methods of Analysis of AOAC INTERNATIONAL* (current edition), AOAC INTERNATIONAL, Rockville, MD, USA.]

2 Applicability

The method for vitamin B_{12} analysis must quantitate multiple forms of vitamin B_{12} individually in a variety of dosage forms in dietary ingredients and dietary supplements (Table 1). The method must also be able to determine active vitamin B_{12} corrinoids individually and distinguish them from inactive forms present in products derived from some microbiological sources.

3 Analytical Technique

Any analytical technique(s) that measures the analytes of interest and meets the following method performance requirements is/are acceptable.

4 Definitions

Active vitamin B_{12}.—For the purposes of this SMPR, active vitamin B_{12} is defined as:

Methylcobalamin.—CAS No. 13422-55-4 (*see* Figure 1).

Table 1. Recommended matrices
Tablets
Capsules
Liquids
Powders
Extracts
Microbial products
Gummies
Softgels
Sublingual forms
Chewables

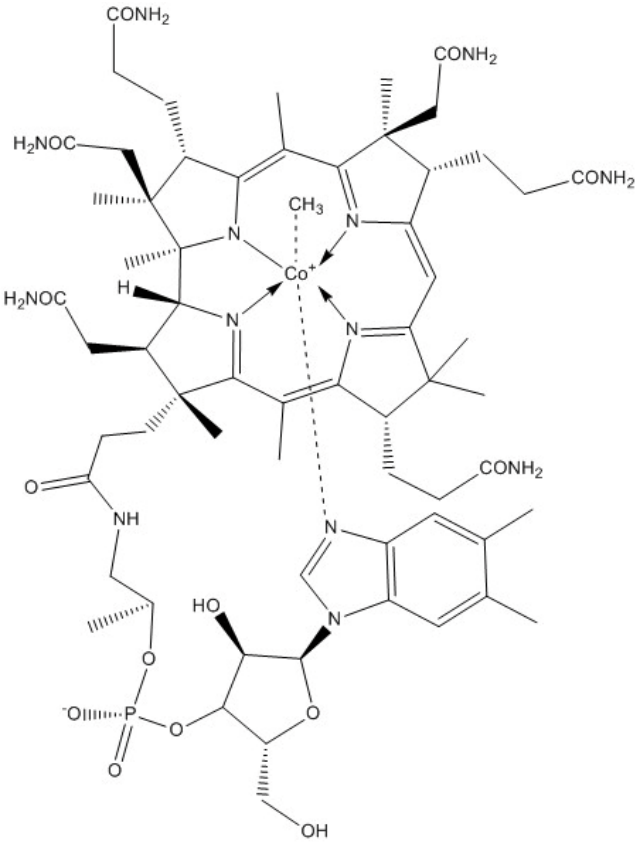

Figure 1. Molecular structure of methylcobalamin.

Cyanocobalamin.—CAS No. 68-19-9 (*see* Figure 2).

Adenosylcobalamin.—CAS No. 13870-90-1 (*see* Figure 3).

Hydroxocobalamin.—CAS No. 13422-51-0 (*see* Figure 4).

Dietary ingredients.—A 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 {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).—Minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.

Quantitative method.—Method of analysis which response is the amount of the analyte measured either directly (enumeration in a mass or a volume), or indirectly (color, absorbance, impedance, etc.) in a certain amount of sample.

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

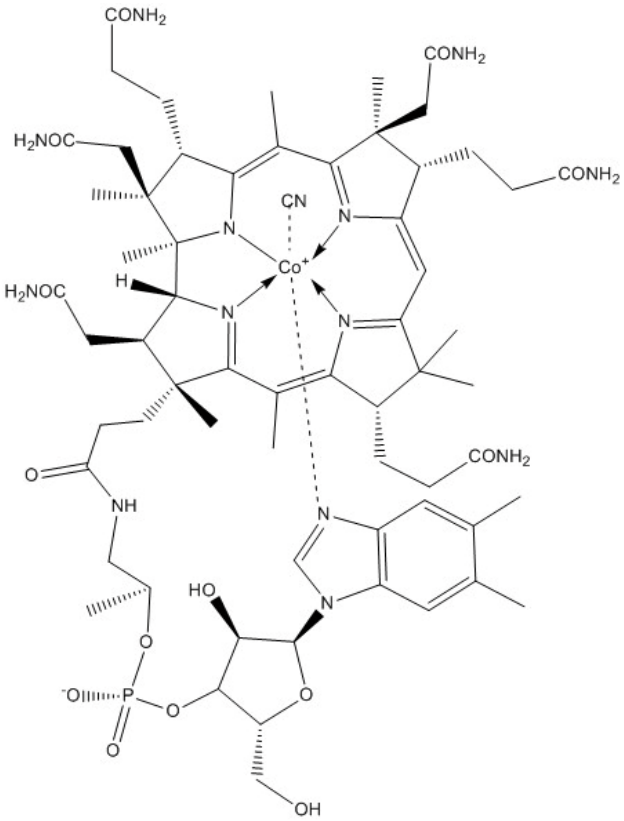

Figure 2. Molecular structure of cyanocobalamin.

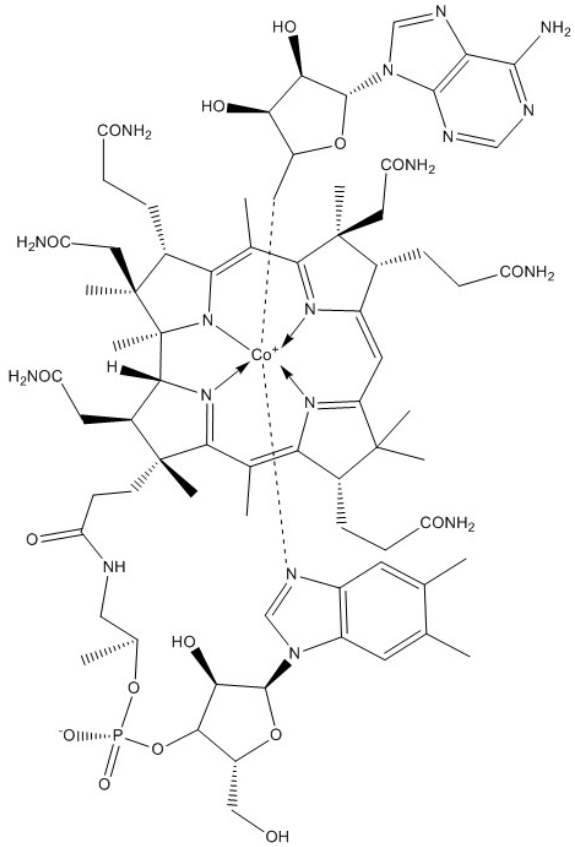

Figure 3. Molecular structure of adenosylcobalamin.

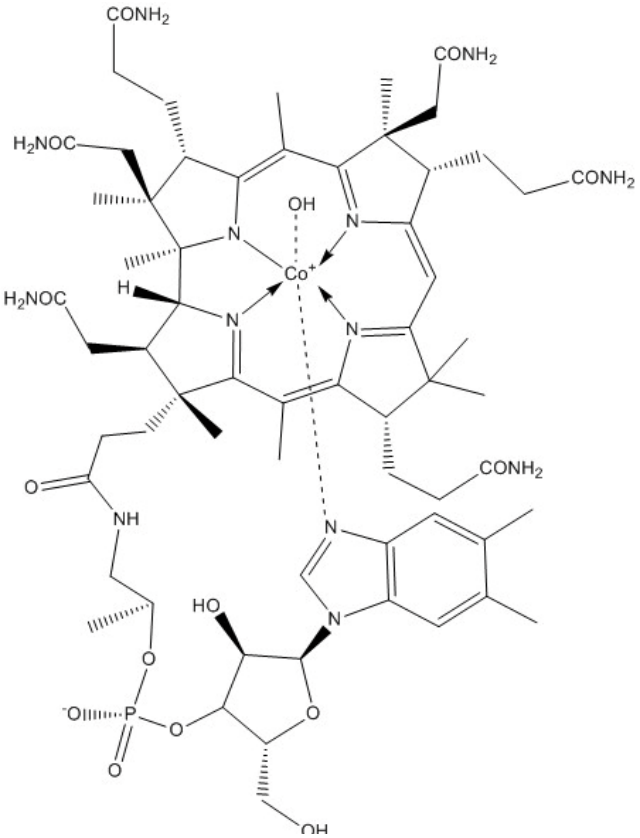

Figure 4. Molecular structure of hydroxocobalamin.

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.

5 Method Performance Requirements

See Tables 2 and 3.

6 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.

7 Reference Material(s)

Refer to Annex F: *Development and Use of In-House Reference Materials* in Appendix F: *Guidelines for Standard Method Performance Requirements*, *Official Methods of Analysis of AOAC INTERNATIONAL* (current edition), AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_f.pdf)

NIST Multivitamin (3280)

NIST Protein Drink Mix (3252)

USP Cyanocobalamin 1152009

USP Methylcobalamin 1424550

Table 2. Analytical range and LOQ requirements	
Analytical range, ppm	0.001 to 1 000 000
Limit of quantitation (LOQ), ppm	≤0.0005

Table 3. Recovery, repeatability, and reproducibility parameters[a]					
Range	<5 ppm	5 to 20 ppm	>20 to 1000 ppm	>0.1 to 1%	>1%
Recovery, %[b]	75–115	80 to 110	95 to 105	97 to 102	98–102
RSD_r, %[b]	≤12	≤8	≤5	≤4	≤2
RSD_R, %[b]	≤20	≤12	≤8	≤6	≤3
[a] Reported as individual vitamin B_{12} analogs.					
[b] % Recovery, $\%RSD_r$, and $\%RSD_R$ shall be determined individually for each claimed matrix.					

USP Hydroxocobalamin HCl 1324319

USP Hydroxocobalamin Acetate 1324308

8 Validation Guidance

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

Appendix F: *Guidelines for Standard Method Performance Requirements*, *Official Methods of Analysis of AOAC INTERNATIONAL* (current edition), AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_f.pdf)

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

9 Maximum Time-to-Result

None.

Approved by the AOAC Stakeholder Panel on Dietary Supplements (SPDS). Final Version Date: September 16, 2016.