**DRAFT AOAC SMPR 2016.XXX; Version 4, June 28, 2016**

# SMPR Name: 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 the 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 method being considered for *Performance Tested Methods* or AOAC *Official Methods of Analysis*, 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* (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA.]

## 2 Applicability:

The method for vitamin B12 analysis must quantitate multiple forms of vitamin B12 individually in a variety of dosage forms in dietary ingredients and dietary supplements. The method must also be able to determine active vitamin B12 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 B12**

For the purposes of this SMPR, active Vitamin B12 is defined as:

Methylcobalamin. CAS number 13422-55-4. See figure 1.
Cyanocobalamin. CAS number 68-19-9 . See figure 2.
adenosylcobalamin. CAS number 13870-90-1. See figure 3.
Hydroxocobalamin. CAS number 13422-51-0. See figure 4.

**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.[1]

[1] 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.

**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**

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.

**5. Method Performance Requirements**:

See table 1 and 2.

**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*, 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_f.pdf

NIST Multivitamin (3280)
NIST Protein Drink Mix (3252)
USP Cyanocobalamin 1152009
USP Methylcobalamin 1424550
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; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_d.pdf

Appendix F: Guidelines for Standard Method Performance Requirements; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_f.pdf

Appendix K: Guidelines for Dietary Supplements and Botanicals; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available on line at: http://www.eoma.aoac.org/app_k.pdf

9. **Maximum Time-To-Result:** None

**Table 1: Analytical Range and LOQ Requirements**

| | |
|---|---|
| Analytical Range (ppm) | 0.001 to 1,000,000 ppm |
| Limit of Quantitation (LOQ) | ≤ 0.0005 ppm |

**Table 2: Recovery, Repeatability, and Reproducibility Parameters**

| **Range** | **<5 ppm** | **5 to 20 ppm** | **>20 to 1000ppm** | **>0.1% to 1%** | **>1%** |
|---|---|---|---|---|---|
| **% Recovery** | 75-115 | 80 to 110 | 95 to 105 | 97 to 102 | 98 – 102 |
| **% RSDr** | <=12 | ≤ 8 | ≤ 5 | ≤ 4 | ≤ 2 |
| **% $RSD_R$** | <=20 | ≤ 12 | ≤ 8 | ≤ 6 | ≤ 3 |
| Reported as individual Vitamin B12 analogs. | | | | | |

**% recovery, % RSDr, and % $RSD_R$ shall be determined individually for each claimed matrix.**

**Table 3: Recommended Matrices**

Tablets
Capsules
Liquids
Powders
Extracts
Microbial products
Gummies
Softgels
Sublingual forms
Chewables

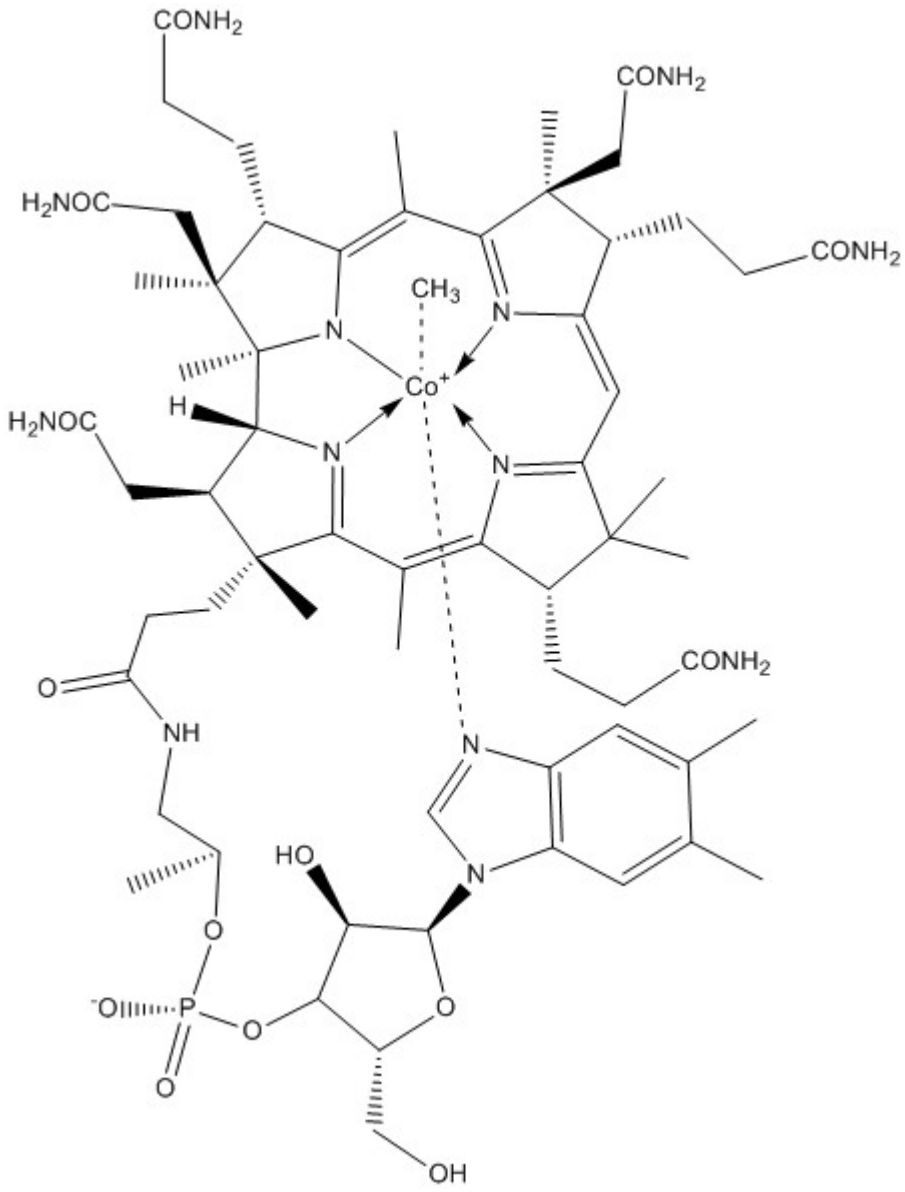


Figure 1: Molecular structure of methylcobalamin.

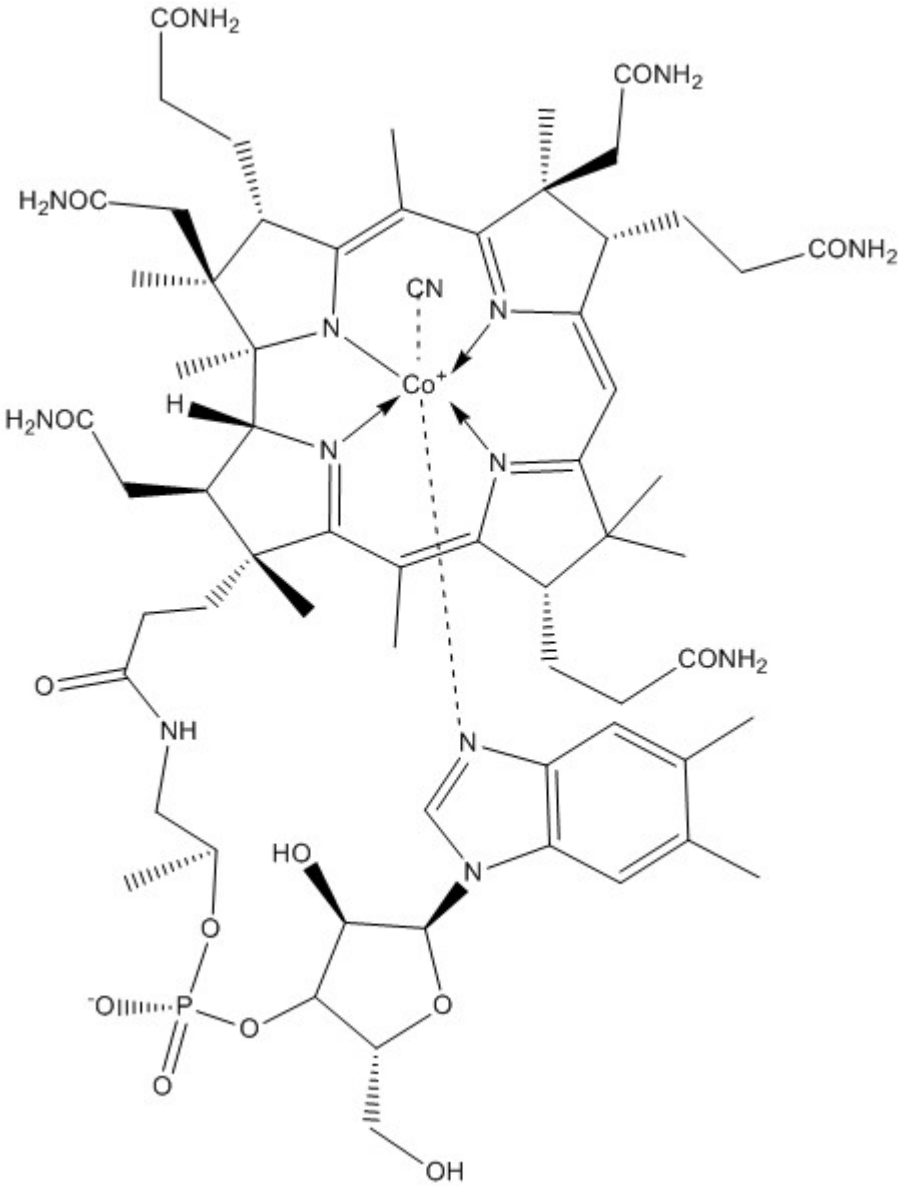


Figure 2: Molecular structure of cyanocobalamin.

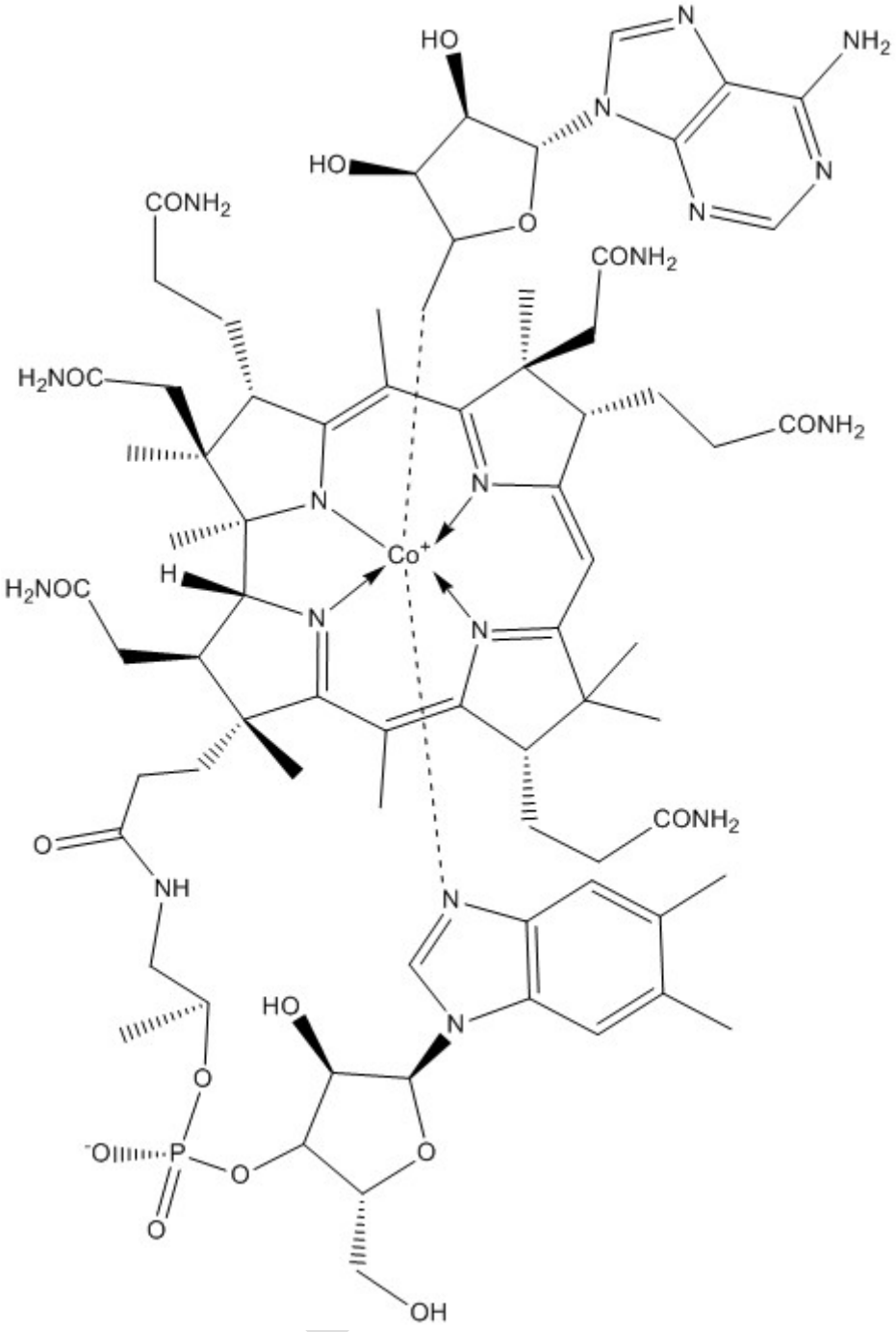


Figure 3: Molecular structure of adenosylcobalamin.

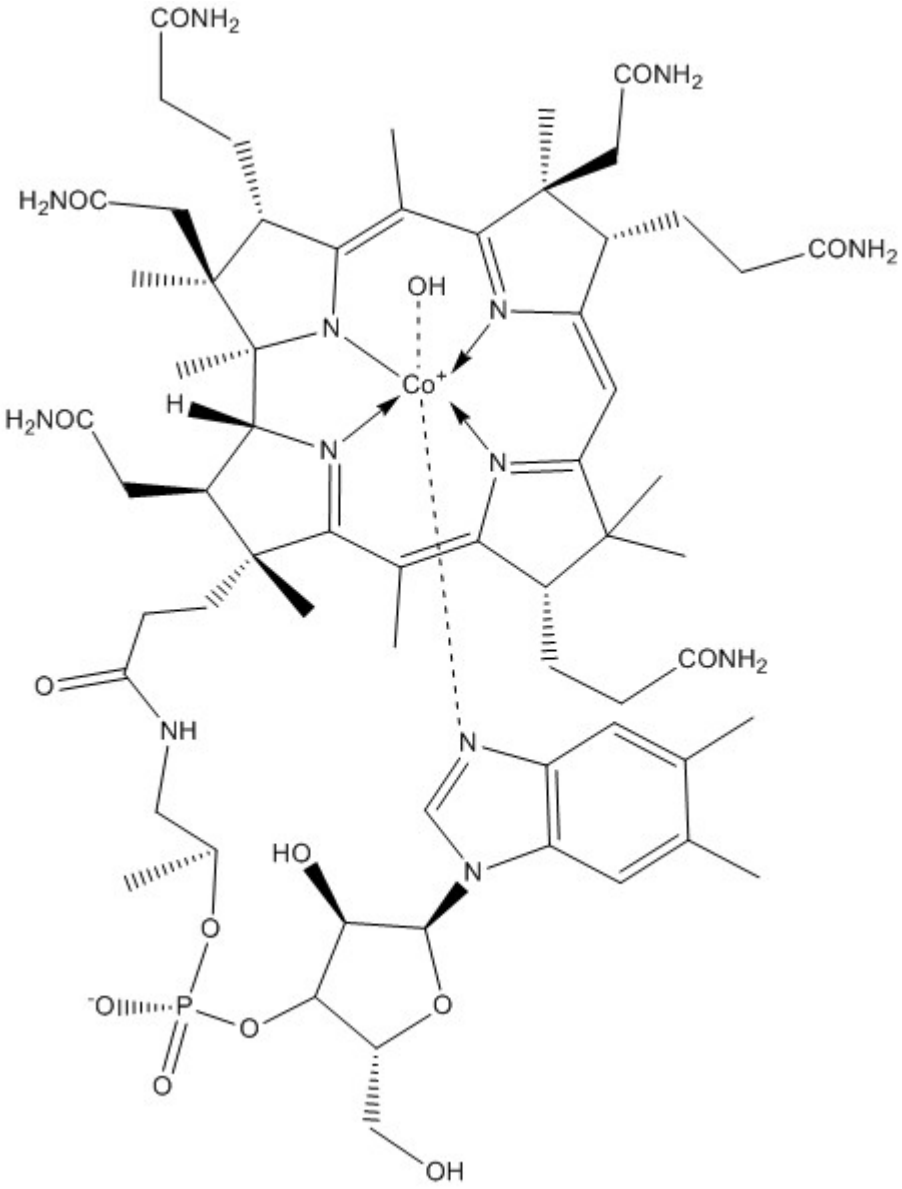


Figure 4: Molecular structure of hydroxocobalamin