

Standard Method Performance Requirements for Vitamin B₁₂ in Infant Formula and Adult/Pediatric Nutritional Formula

Intended Use: Global Dispute Resolution Method

1 Applicability

Determination of vitamin B₁₂ in all forms of infant, adult, and/or pediatric formula (powders, ready-to-feed liquids, and liquid concentrates). For the purpose of this SMPR, vitamin B₁₂ is defined as cobalt-containing corrinoids with the biological activity of cyanocobalamin such as cyanocobalamin (CAS 68-19-9), aquocobalamin (CAS 13422-52-1), hydroxycobalamin (CAS 13422-51-0), methylcobalamin (CAS 13422-55-4), and adenosylcobalamin (CAS 13870-90-1).

2 Analytical Technique

Any analytical technique that meets the following method performance requirements is acceptable.

3 Definitions

Adult/pediatric formula.—Nutritionally complete, specially formulated food, consumed in liquid form, which may constitute the sole source of nourishment (AOAC SPIFAN, 2010), made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

Infant formula.—Breast-milk substitute specially manufactured to satisfy, by itself, the nutritional requirements of infants during the first months of life up to the introduction of appropriate complementary feeding (Codex Standard 72-1981), made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

Limit of detection (LOD).—The minimum concentration or mass of analyte that can be detected in a given matrix with no greater than 5% false-positive risk and 5% false-negative risk.

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 SD or RSD calculated from among-laboratory data; expressed as the reproducibility 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.

Analytical range	0.01–5.0 ^b	
Limit of detection (LOD)	≤0.004 ^b	
Limit of quantitation (LOQ)	≤0.01 ^b	
Repeatability (RSD _r)	0.01–0.2 ^b	≤15%
	>0.2 ^b	≤7%
Recovery	90–110%	
Reproducibility (RSD _R)	≤11%	
^a Concentrations apply to (1) “ready-to-feed” liquids “as is”; (2) reconstituted powders (25 g into 200 g water); and (3) liquid concentrates diluted 1:1 by weight.		
^b µg/100 g expressed as cyanocobalamin in reconstituted final product.		

4 Method Performance Requirements

See Table 1.

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.

6 Reference Material(s)

National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 1849 Infant/Adult Nutritional Formula, or equivalent. The SRM is a milk-based, hybrid infant/adult nutritional powder prepared by a manufacturer of infant formula and adult nutritional products. A unit of SRM 1849 consists of 10 packets, each containing approximately 10 g of material. Reference value of vitamin B₁₂ in NIST 1849 is 0.04 (±0.08) mg/kg vitamin B₁₂. NIST reference values represent a best estimate of the true value where all known or suspected sources of bias have not been fully investigated. Reference values have associated uncertainties that may not include all sources of uncertainty and may represent only a measure of the measurement method’s precision [Sharpless, K.E., Lindstrom, R.M., Nelson, B.C., Phinney, K.W., Rimmer, C.A., Sander, L.C., Schantz, M.M., Spatz, R.O., Thomas, J.B., Turk, G.C., Wise, S.A., Wood, L.J., & Yen, J.H. (2010) *J. AOAC Int.* 93, 1262–1274].

7 Validation Guidance

Recommended level of validation: *Official Methods of Analysis*SM.

8 Maximum Time-to-Signal

No maximum time.

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