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Infant formula and adult nutritionals — Determination of vitamin B_{12} by reversed phase high performance liquid chromatography (RP-HPLC)

Formules infantiles et produits nutritionnels pour adultes — Détermination de la teneur en vitamine B_{12} par chromatographie liquide haute performance en phase inverse (CLHP-PI)

Reference number
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Foreword

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 34, *Food products* in collaboration with AOAC INTERNATIONAL. It is being published by ISO and separately by AOAC INTERNATIONAL. The method described in the International Standard is equivalent to the AOAC Official Method 2011.10: *Vitamin B_{12} in infant and pediatric formulas and adult nutritionals*.

Infant formula and adult nutritionals — Determination of vitamin B_{12} by reversed phase high performance liquid chromatography (RP-HPLC)

WARNING — The use of this International Standard can involve hazardous materials, operations and equipment. This International Standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this International Standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

1 Scope

This International Standard specifies a method for the quantitative determination of vitamin B_{12} in infant and adult formula (powders, ready-to-feed liquids and liquid concentrates) by reversed phase high performance liquid chromatography.

2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1
vitamin B_{12}
cyanocobalamin and other cobalt-containing corrinoids with vitamin B_{12} biological activity, such as aquocobalamin, hydroxycobalamin, methylcobalamin and adenosylcobalamin, converted to cyanocobalamin

2.2
adult nutritional
nutritionally complete, specially formulated food, consumed in liquid form, which may constitute the sole source of nourishment, made from any combination of milk, soy, rice, whey, hydrolysed protein, starch and amino acids, with and without intact protein

2.3
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

[SOURCE: Codex Standard 72-1981]

3 Principle

Cyanocobalamin and other cobalt-containing corrinoids are extracted from the sample using sodium acetate buffer (pH = 4,5) and the latter converted to cyanocobalamin using potassium cyanide at 105 °C. Extracts are purified and concentrated with C8 or C18 solid-phase extraction (SPE) cartridges and analysed with size-exclusion and reversed-phase chromatography. Determination of vitamin B_{12} is made by liquid chromatography with visible detection at 550 nm.

4 Reagents and materials

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity.

4.1 Glacial acetic acid.

4.2 Acetonitrile, HPLC grade.

4.3 Drierite, desiccant, anhydrous calcium sulfate, 8 mesh.

4.4 Ethanol, denatured.

4.5 Formic acid, 88 %.

4.6 Potassium cyanide, 97 %.

4.7 Riboflavin, 98 % to 102 % purity.

4.8 Sodium acetate anhydrous or sodium acetate trihydrate, ACS.

4.9 Taka-Diastase, Accurate Chemical Co.[1)] or equivalent.

4.10 Triethylamine, HPLC grade.

4.11 Vitamin B_{12} (cyanocobalamin) primary reference standard, e.g. USP Reference 1152009 (approximately 10 µg/mg), Official lot[1)]. Store in a desiccator protected from white light.

4.12 Preparation of solutions and standard solutions

4.12.1 General

All solutions may be scaled up or down for convenience provided good laboratory practices are observed. Solutions can be stored refrigerated or at ambient temperature in tight, inert containers unless otherwise specified.

4.12.2 Preparation of solutions

4.12.2.1 HPLC mobile phase A. Dilute 4,0 ml of triethylamine with 1 000 ml of water. Adjust the pH to 5 to 7 with approximately 1,25 ml concentrated formic acid (4.5). Expiration: 1 week.

4.12.2.2 HPLC mobile phase B. Mix 4,0 ml of triethylamine and 250 ml of acetonitrile with 750 ml of water. Adjust the pH to 5 to 7 with approximately 1,25 ml concentrated formic acid. Expiration: 1 week in tightly stoppered container.

4.12.2.3 HPLC mobile phase C. Mix 4,0 ml of triethylamine and 750 ml of acetonitrile with 250 ml of water. Adjust the pH to 5 to 7 with approximately 1,25 ml concentrated formic acid. Expiration: 1 week in tightly stoppered container.

4.12.2.4 HPLC mobile phase D. Dilute 50 ml of acetonitrile to 2 000 ml with water. Expiration: 1 week in tightly stoppered container.

4.12.2.5 Mixture of acetonitrile and water, volume fraction 10 %. Dilute 150 ml of acetonitrile to 1 500 ml with water. Expiration: 1 month in tightly stoppered container.

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1) This is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

4.12.2.6 Mixture of acetonitrile and water, SPE elution solvent, volume fraction 30 %. Dilute 30 ml of acetonitrile to 100 ml with water. Expiration: 1 month in tightly stoppered container.

4.12.2.7 Mixture of acetonitrile and water, column cleaning and storage solution, volume fraction 50 %. Dilute 500 ml of acetonitrile to 1 000 ml with water in a volumetric flask. Expiration: 6 months.

4.12.2.8 Mixture of ethanol and water, volume fraction 25 %. Dilute 50 ml of ethanol to 200 ml with water. Expiration: 1 year in tightly stoppered container.

4.12.2.9 Potassium cyanide solution, mass concentration ρ = 4 g/l. Dissolve 0,02 g of potassium cyanide in and dilute to 5 ml with sodium acetate buffer (4.12.2.11) substance concentration c = 0,25 mol/l). Prepare fresh immediately before use.

4.12.2.10 Potassium cyanide solution, ρ = 10 g/l. Dissolve 0,25 g of potassium cyanide in water and dilute to 25 ml. Prepare fresh immediately before use.

4.12.2.11 Sodium acetate buffer, c = 0,25 mol/l. Dissolve 41,0 g of sodium acetate anhydrous or 68,0 g of sodium acetate trihydrate in approximately 1 800 ml of water. Adjust the pH to 4,5 with concentrated acetic acid (approximately 40 ml). Dilute to 2 000 ml with water. Expiration: 3 months.

4.12.2.12 Resolution test solution. Weigh approximately 0,005 g of riboflavin onto a weigh paper. Transfer to a 100 ml volumetric flask and bring to volume with 10 % acetonitrile solution Stir to dissolve. Mix equal amounts of solution with the highest concentration of vitamin B_{12} working standard solution. Expiration: 1 week.

4.12.2.13 Taka-Diastase solution, ρ = 60 g/l. Dissolve 0,6 g of Taka-Diastase in 10 ml of water. Prepare fresh daily before use.

4.12.3 Preparation of standard solutions

4.12.3.1 General. Prepare all standard solutions under UV shielded fluorescent lights and store at 2 °C to 8 °C in tightly stoppered volumetric flasks.

4.12.3.2 Vitamin B_{12} stock standard solution, ρ = 10 000 µg/l. Accurately weigh the appropriate amount of vitamin B_{12} standard (4.11) to give a stock standard concentration of 10 000 µg/l. Dissolve in and dilute to 100 ml with 25 % ethanol (4.12.2.8). Expiration: 6 months.

Calculate the amount of vitamin B_{12} standard to be weighed, m_w, in milligrams using Formula (1):

$$m_w = 10\,000 \times 0,1 \times \frac{1}{P} \quad (1)$$

where

10 000 is the desired stock standard solution concentration, in µg/l;

0,1 is the dilution volume, in l;

P is the purity of the vitamin B_{12} standard (4.11) in µg of cyanocobalamin per mg of standard.

4.12.3.3 Vitamin B_{12} intermediate standard solution, ρ = 1 000 µg/l. Dilute 10 ml of stock standard solution (4.12.3.2) to 100 ml with water. Expiration: 1 week.