Vitamin B_{12} - A review of analytical methods for use in food

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Vitamin B12 - A review of analytical methods for use in food

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Abstract

Vitamin B_{12} - a water-soluble vitamin that is found naturally in some foods, added to others, and is available as a dietary supplement - plays an important role in the functioning of the brain and nervous system, and in the formation of red blood cells. The requirement for vitamin B_{12} is low and the storage capacity in humans is high, therefore deficiency is rare in healthy populations. However certain population groups, such as infants, the elderly, vegetarians or vegans, can be prone to deficiency. This can be controlled in vegetarian groups by supplementation. Deficiency, particularly in the elderly, is often a result of inadequate metabolism and is not related to dietary intake; the need for analysis of foods is therefore low. In specific population groups, such as vegetarians, intake may be reliant on supplementation and in others, such as infants, fortification of infant formula and follow-on foods is important. Analysis may be required in fortified foods or supplements, primarily to confirm label declarations. Methods for the analysis of vitamin B_{12} include microbiological assay, polarographic, spectrophotometric, radio-ligand binding and various chromatographic techniques. Microbiological assay has been the most commonly used assay technique for foods and has been used for the majority of available food nutrition datasets. LGC has extensive experience of the determination of vitamin B_{12} using an in-house microbiological assay procedure although this technique is no longer supported. In this report, we review methods for the analysis of vitamin B_{12} and describe methods that may be applied in the absence of a supported microbiological assay.

Contents

Introduction

Vitamin B_{12} is a water-soluble vitamin that is found naturally in some foods, is added to others, and is also available as a dietary supplement. In fact, vitamin B_{12} exists in several forms, all of which contain cobalt and are called “cobalamins”. Vitamin B_{12} is commonly called cyanocobalamin; however the name can refer to any of the cobalamins that exhibit similar biological activity. Methylcobalamin and 5-deoxyadenosylcobalamin are the forms of vitamin B_{12} that are active in human metabolism. Cyanocabalamin and hydroxycobalamin are used pharmacologically with cyanocobalamin primarily being used for fortification.

Vitamin B_{12} plays an important role in the functioning of the brain and nervous system, and in the formation of red blood cells. The requirement for vitamin B_{12} is low and the storage capacity in humans is high therefore deficiency is rare in healthy populations. Certain population groups such as infants, the elderly, vegetarians or vegans can be prone to deficiency. Severe deficiency, primarily caused by malabsorption or metabolic failure, causes pernicious anemia, a failure to produce sufficient red blood cells or haemoglobin. This disease, primarily of the elderly, can be treated by vitamin B_{12} injections but not cured. Vitamin B_{12} is very important for the development and growth of children and infant formula is an important vehicle for fortification.

This report summarises a review of analytical methods for the determination of vitamin B_{12} in food. Mention of a commercial analytical product in this report does not imply endorsement by the Government Chemist.

Dietary sources of vitamin

Vitamin $B_{12,}$ occurring naturally in food, derives from bacterial synthesis and therefore occurs almost exclusively in foods of animal origin. Small amounts have been determined in certain plant sources such as seaweeds and algae but are not all available for human consumption and digestion. Cobalamins are usually bound to other food components, especially proteins, and must undergo enzymatic and acid hydrolysis in the gut to be released. Similar processes are required for in-vitro analysis.

Dairy foods, milk and milk products, eggs, meat and seafoods are the main natural sources in the diet. Vitamin preparations or supplements containing vitamin B_{12} are widely available and foods, such as breakfast cereals, are often fortified with vitamin B_{12}. The capacity to utilise vitamin B_{12} however, depends upon the availability of “intrinsic factor”, a glycoprotein

required for its absorption. Intake cannot therefore be used as a sole measure of nutritional status. Infant formula and follow-on foods are an important area for fortification.

Stability

Cyanocobalamin is the most stable form of vitamin B_{12} and is normally used for fortification. It is most stable at pH 4 - 4.5 and to heat, including autoclaving between pH 4 and 7. Crystalline forms are stable when protected from light. Severe acid or alkali, strong light or the presence of oxidising agents will however destroy the vitamin. Excess cyanide will displace other moieties from the cobalt beta position to form cyanocobalamin.

Analytical requirements

Nutritional status is determined by analysis of plasma or serum concentrations of cyanocobalamin or related forms and metabolites. Since deficiency is rare and often not related to dietary intake, the need for analysis of foods is low. The exception is in specific population groups where intake may be reliant on supplementation or fortification. Since the requirement for vitamin B_{12} is small and there is no risk associated with excessive intake, the need for regulation in this area is limited. Analysis may be required in fortified foods or supplements, primarily to confirm label declarations.

Extraction

When synthetic vitamin B_{12} is added to fortified foods and dietary supplements, it is already in free form and does not require vigorous extraction. Simple aqueous extraction can be carried out, usually at a pH of around 4. Heating may be used and clean-up may be required to remove other analytical components.

For natural foods, vitamin B_{12} is bound to protein and must be released before analysis. This is often accomplished simply by denaturing the protein by heating during the extraction, although pepsin or other protease enzymes may also be used. For starchy foods, amylase may also be added to help break down the food matrix. Excess cyanide is added when determining natural vitamin B_{12} to convert all of the cobalamins to the cyanocobalamin form.

Analysis of vitamin B_{12} at natural levels in foods is difficult as it is normally present at very low concentration and may be accompanied by much higher levels of other food

components which may interfere with subsequent analysis. These need to be removed before quantitation of the vitamin B_{12}.

Analytical methods

Methods for the analysis of vitamin B_{12} include microbiological assay, polarographic, spectrophotometric, radio-ligand binding and various chromatographic techniques. For clinical use, radio-ligand binding assays are commonly used but these are not normally suitable for use in foods.

Microbiological assay has been the most commonly used assay technique for foods and has been used for the majority of available food table data. It relies on the specific requirement for vitamin B_{12} by certain bacterial organisms (e.g. *Lactobacillus delbreueckii*) to enable their growth in a supporting medium. Under appropriate conditions, the amount of growth obtained is proportional to the amount of vitamin B_{12} in the test extract. Such assays can be considered routine for vitamin B_{12} but require overnight incubation, can be subject to contamination and require considerable laboratory set-up and maintenance time. The sensitivity is high enabling the detection of low concentrations of vitamin B_{12} but other food components may interfere with the determination.

The development of commercial test kits for the analysis of water-soluble vitamins in foods using microbiological assay (e.g. VitaFast® kits) has simplified these procedures. However careful use is still required and the extraction procedures given in the kit may need further validation depending on the samples to be analysed.

Official methods

There are few official methods for vitamin B_{12} in food. The following methods are available:

USP/BP:

Determination of cyanocobalamin and hydroxocobalamin in pure substance, injectable solutions and oil or water soluble vitamins tablets and capsules. – Spectrophotometric and HPLC methods with specific scope as above.

AOAC:

There are a number of approved procedures within AOAC although almost all of these are for the analysis of infant formula and related nutritional products as below. The more recent methods were developed under the AOAC Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN) project.

AOAC 952.20 Vitamin B_{12} in vitamin preparations - Microbiological assay
AOAC 986.23 Vitamin B_{12} in milk-based infant formula - Microbiological assay
AOAC 2011.08 Vitamin B_{12} in infant formula and adult nutritionals - HPLC-UV with immunoaffinity extraction (1st action)
AOAC 2011.09 Determination of vitamin B_{12} in infant formula and adult nutritionals using HPLC after purification on an immunoaffinity column (1^{st} Action) – HPLC with immunoaffinity extraction

Note: For final action status, AOAC recommended combining 2011.08 with 2011.09 as they are very similar. SPIFAN, however, rejected both of these as they use a proprietary (single-source) IAC cartridge and are proceeding with AOAC 2011.10.

AOAC 2011.10 Determination of vitamin B_{12} in infant formula and adult nutritionals by HPLC: (1^{st} Action) – HPLC with SPE clean-up (column switching)
AOAC 2011.16 Determination of vitamin B_{12} in infant formula and adult nutritionals by surface plasmon resonance: (test kit method) – Biacore technology (1st action)

ISO: There are no specific methods for vitamin B_{12} but the recommended CODEX procedures refer to the AOAC microbiological methods 952.20 and 986.23.

CEN / BS: There are no approved methods for vitamin B_{12}

Commercial methods:

R-Biopharm test kits: VitaFast® test kit: 96 well microtiter plate-based microbiological assay. These are produced by R-Biopharm and provide a relatively simple means of applying the microbiological assay without the need for in-house maintenance of organisms etc. The B_{12} test kit is described for the determination of vitamin B_{12} at natural or added levels in food, feed and pharmaceutical products.

EASI-EXTRACT® VITAMIN B_{12}: Monoclonal antibody based immunoaffinity columns for use in conjunction with an HPLC or LC-MS/MS system for detection of vitamin B_{12} in foods and similar matrices. The columns (available in two sizes) are described for the selective extraction of vitamin B_{12} before analysis using HPLC or LC-MS. The conditions required for HPLC with UV detection are given.

Note: As used in the AOAC methods above. The extraction is mainly validated for infant formulae and some additional validation may be required for other foods.

Ridascreen®Fast Vitamin B_{12}: Competitive enzyme immunoassay for the quantitative analysis of vitamin B_{12} in fortified food, feed and vitamin products.

HPLC or LC-MS methods

There are few published procedures for vitamin B_{12} in food apart from those discussed above. Published procedures are similar to the AOAC methods and are mainly for fortified products. HPLC with UV detection is difficult because of the low level of vitamin B_{12} in unfortified foods and relatively high detection limits with UV detectors. LC-MS procedures are rare at present although some conditions and multivitamin methods including vitamin B_{12} have been published as below. These provide a useful starting point if mass spectrometry is desired but further validation would be necessary.

Table 1: Mass spectrophotometric methods for Vitamin B_{12}

Analyte	Technique	Reference
Vitamin B_{12} standard	LC-MS	1
Vitamins B_5,B_8,B_9 & B_{12} in fortified infant formula	UPLC / Triple Quad MS	2
Water soluble vitamins in various foods	LC/ESI-MS\MS	3
Water soluble vitamins in fortified beverages & supplements	UPLC/MS/MS	4

Methods used at LGC

LGC has long experience of the determination of vitamin B_{12} using an in-house microbiological assay procedure, although this technique is no longer supported. The VitaFast® test kit is not used routinely although similar kits are used successfully for other water soluble vitamins.

Recent analysis has focussed on the HPLC-UV procedure with immunoaffinity cartridge extraction. The R-Biopharm test kit is used for this purpose. It has been successfully used for infant formulae and baby foods (fruit, pasta and cereal). The method performs well although chromatographic problems can be encountered since the cartridges have a low maximum retention capacity which limits the extent to which sample extracts can be

concentrated. Additional validation is required for foods other than those described in the test kit instructions (mainly infant formula).

A method using aqueous extraction followed by ion-pair HPLC with UV detection has also been used for analysis of vitamin premixes but is only suitable for this purpose.

Conclusion

Vitamin B_{12} - a water-soluble vitamin that is found naturally in some foods, added to others, and is available as a dietary supplement - plays an important role in the functioning of the brain and nervous system, and in the formation of the red blood cells. Vitamin B_{12} deficiency is rare in healthy populations. However certain population groups, such as infants, the elderly, vegetarians or vegans, can be prone to deficiency. This can be controlled in vegetarian groups by supplementation. Deficiency, particularly in the elderly, is often a result of inadequate metabolism and is not related to dietary intake; the need for analysis of foods is therefore low. However analytical methods are required for fortified foods or supplements, primarily to confirm label declarations. Methods for the analysis of vitamin B_{12} include microbiological assay, polarographic, spectrophotometric, radio-ligand binding and various chromatographic techniques. Microbiological assay has been the most commonly used assay technique for foods and has been used for the majority of available food nutrition datasets. However such techniques are expensive to support in the absence of a minimum level of use. There are other methods for the analysis for vitamin B_{12} reviewed above that may be applied in the absence of a supported microbiological assay.

References

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