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RECENT TRENDS IN THE QUANTIFICATION OF VITAMIN B

(Kaedah Terkini dalam Pengkuantitian Vitamin B)

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Abstract

Numerous analytical methods have been developed for a variety of matrices to quantify vitamin B compounds. It is important to establish a method with great accuracy and precision of chromatographic separation. This review provides a wide-ranging search of the vitamin B extraction methods developed during the last five years in all applications. Particular emphasis is given to sample extraction and chromatography analysis for vitamin B compounds' extraction in different applications such as biological fluids, food, and pharmaceuticals. Hence, this review will elaborate the properties of vitamin B and its metabolites, and the extraction and quantification of water-soluble vitamin B content in foods for compliance monitoring and for their particular application.

Keywords: review, sample preparation, extraction, vitamin B, chromatography analysis

Abstrak

Terdapat pelbagai kaedah analitikal telah digunakan untuk menentukan kandungan vitamin B dalam pelbagai sampel matriks. Ia adalah penting untuk membangunkan sesuatu kaedah yang mempunyai ketepatan dan kejituan yang tinggi dalam pemisahan kromatografik. Ulasan ini mengandungi carian menyeluruh untuk semua kaedah penentuan vitamin B yang telah dibangunkan sepanjang lima tahun terkini dalam semua aplikasi. Fokus diberikan kepada analisis pengekstrakan sampel dan analisis kromatografi untuk sebatian vitamin B dalam pelbagai aplikasi seperti cecair biologi, makanan, dan farmaseutikal. Oleh itu, ulasan ini akan menjelaskan ciri-ciri vitamin B dan metabolitnya, dan pengekstrakan dan pengkuantitian kandungan vitamin B larut air bagi tujuan pemantauan pematuhan kepada peraturan dan juga aplikasi tertentu.

Kata kunci: ulasan, penyediaan sampel, pengekstrakan, vitamin B, analisis kromatografi

Introduction

Vitamins B compounds differ in chemical structure and biological roles, which are essential for health. The deficiency of vitamin B, will have negative consequences for the catabolic process because one or more vitamin B compounds are involved in every aspect of the essential process for generating energy within cells [1]. A study showed that vitamin B performed essential and closely interrelated roles in cellular functioning, acting as co-enzymes in a vast array of catabolic and anabolic enzymatic reactions [2]. Typically, it is synthesised by plants, which is in chloroplast, mitochondria, and cytosol that are regulated to the plant's fluctuating requirements. On the other hand, there is and exception for vitamin B_{12} which is synthesised by bacteria and is typically sequestered from animal-derived food. Although most vitamins are derived from plants, they are often consumed indirectly from the higher up food chain in foods of animal origin including meat, dairy, and eggs [3]. Most experts agreed that vitamin B concentration between 200 and 900 ng/mL is considered sufficient, whereas a concentration below 200 ng/mL indicates insufficiency. Daily intake of 4 to 7 µg dietary vitamin B_{12} is associated with sufficient concentrations of vitamin B_{12} related as biomarkers in the healthy and young population.

Early detection of vitamin B deficiency is of key importance to identify affected individuals who can then receive adequate and appropriate treatment. Therefore, there are a lot of methods have been used in previous studies to determine vitamin B compounds such as UV-Visible spectrophotometry, chemiluminescence, fluorimetry, microbiological, capillary electrophoresis, and high performance liquid chromatography (HPLC). Besides, a few comprehensive reviews of pre-treatment and determination of vitamins had been published. The most common sample preparation methods such as solid-phase extraction, ultrasonic-assisted extraction (UAE), supercritical fluid extraction (SFE), liquid–liquid extraction (LLE), and dispersive liquid–liquid microextraction (DLLME) using different analysis methods such as chromatography, immunoassay, electrophoretic methods, and biosensors for analysis of vitamins since 2010 had been reviewed [4]. Therefore, this review includes method development in different matrices including food, plants, biological fluids, and pharmaceutical preparations published during the last five years. Emphasis was given to various vitamin B compounds that were measured including sample preparation and extraction methods developed. Hence, this review will provide a valuable addition to the literature and will enable other researchers to access developments across several applications and to select or develop the optimal method for their particular application. This review on all vitamin B extraction methods includes comprehensive databases from previous literature which has been published from 2015 to 2020.

Vitamin B metabolites

Vitamins represent a group of various compounds, where they may be present in several chemically-diversed but biologically interconvertible forms. Besides, they are essential dietary components for humans and animals. They are needed in relatively small amounts to maintain good health and sustain life [5]. Therefore, lack of a sufficient amount of vitamins can lead to serious illnesses. Furthermore, the amount of vitamins that is required for normal development and maintenance of body functions is not always contained in the human diet and it might be lost through chemical reactions or even by extraction and leaching during storage and processing of food which is often the case with water-soluble vitamins [6]. Thus, to confirm the percentage of the recommended dietary allowance (RDA) which present in the final food products, a rapid, efficient, and reliable analytical determination of the vitamin content in food is needed for manufacturers, food laboratories, and also regulatory authorities. The B-group is a big family which consists of vitamins B_1 (thiamine), B_2 (riboflavin), B_3 (niacin), B_5 (pantothenic acid), B_6 (pyridoxine), B_8 (biotin), B_9 (folic acid), and B_{12} (cyanocobalamin). Several vitamin B compounds play significant roles and act mainly as coenzymes to produces energy. The biological roles, dietary sources, RDA, and chemical structures of vitamin B_1, B_2, B_3, B_5, and B_{12} are presented in Table 1.

Table 1. The biological roles, dietary sources, RDA, and chemical structures of vitamin B_1, B_2, B_3, B_5, and B_{12}. [2,4]

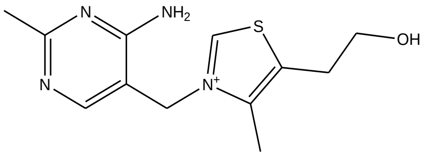
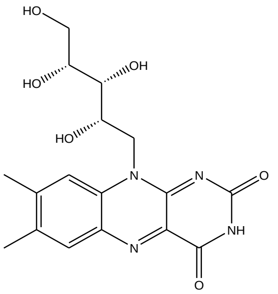
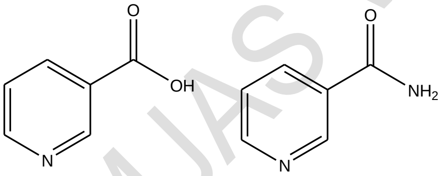
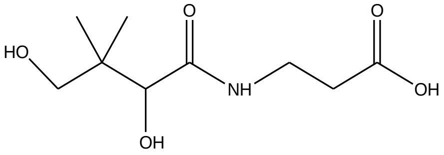
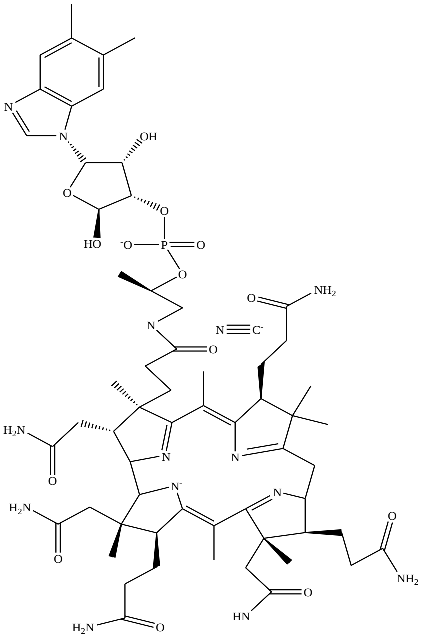
Vitamin B Forms	**Benefits**	**Dietary source**	**RDA**
Thiamine (B_1)  	Thiamine is a portion of an enzyme that is required for energy metabolism and significant for nerve function.	Moderate amounts in all nutritious foods: wholegrain/enriched cereals, breads, nuts, and seeds.	1.2/1.1 mg
Riboflavin (B_2)  	Riboflavin is a portion of an enzyme that is required for energy metabolism and important for skin health and normal vision.	Enriched, wholegrain cereals and breads, milk products, and leafy green vegetables.	1.3/1.1 mg
Niacin (B_3)  	Niacin is a portion of an enzyme that is required for energy metabolism and important for digestive, nervous systems, and skin health.	Fish, meat, peanut butter, asparagus, and also mushrooms.	16/14 mg
Pantothenic acid (B_5)  	Pantothenic acid is a portion of an enzyme that is required for energy metabolism.	It is widespread in foods.	5 mg

Table 1 (cont'd). The biological roles, dietary sources, RDA, and chemical structures of vitamin B_1, B_2, B_3, B_5, and B_{12}. [2,4]

Vitamin B Forms	**Benefits**	**Dietary source**	**RDA**
Cyanocobalamin (B_{12}) 	Cyanocobalamin is a portion of an enzyme required for the production of new cells and important to the function of nerves.	Milk, eggs, seafoods, meats, and not present in plant foods.	2.4 µg

Sample pre-treatment methods

Analysing water-soluble compounds such as vitamin B compounds provides a great challenge to ensure an efficient extraction followed by sample clean-up to remove the interfering substances prior to analyte separation and/or detection. Thus, sample preparation is crucial to allow the detection of compounds especially at a low concentration level in a complex matrix. During the sample preparation process, different substances can be pre-concentrated and separates, thus able to improve the analytical performance such as accuracy, sensitivity, and also selectivity. Besides, many effective sample preparation technologies have emerged and give advantages such as reduce the personnel costs and time consumption.

The sample preparation method will be depending on the type of sample matrix used, either liquid or solid samples [4]. In brief, the extraction process is basically based on enzymatic treatment or acid hydrolysis and protein precipitation to extract the analyte of interest from the other complex matrix.

Solid-phase extraction

Solid-phase extraction (SPE) is among the commonly used methods for the extraction of vitamin B in aqueous sample [4]. Conventional SPE procedure consists of four basic steps which are conditioning, sample adsorption, washing, and elution. However, the extraction and clean-up of analytes in samples using this type of SPE is time-consuming and error-prone. Therefore, an improvement method called online solid-phase extraction liquid chromatography (online SPE-

LC) is getting more attention. Online SPE follows the same steps and is governed by the same principles in extraction and clean-up process. In online SPE-LC, the eluted analytes are directly transferred into the analytical column for separation and quantitation [7]. Thus, the online SPE method has the advantages of the significant reduction in sample preparation time and human handling which leads to high sensitivity and accuracy of the produced result. Besides, online SPE has advantages of which the column can be washed and reuse due to the high-pressure pump. Thus, a cycle of SPE column's enrichment, impurity removal, and column equilibrium could be achieved consistently [7].

Online SPE techniques have made faster methods possible by increasing sample throughput. Besides, other important advantages of online coupling techniques are the ability to eliminate analyte losses by evaporation or degradation during sample preconcentration, improve precision and accuracy, and also reduce risks of contamination of samples and sample extracts [7]. Furthermore, higher sensitivity also can be achieved due to the transfer and analysis of the entire extracted sample on the analytical system. It was reported that smaller sample volumes sufficiently sensitive to analyse large number of compounds with lower limit of detection (LOD) [4]. On the other hand, online SPE consumes smaller amount of solvents compared to conventional SPE, thus reduces the cost of organic solvents waste disposal. Besides, SPE coupled online to HPLC does not require all residual water to be removed from cartridges due to the elution solvents used are compatible with the liquid chromatography (LC) separation methods [8].

Usually, a method based on SPE is used as preconcentration and clean-up step before the determination of vitamin B. A SPE based on the dispersion of a solid sorbent in liquid samples which called dispersive solid-phase extraction (d-SPE) is a more environmental, economical, and faster method compared to conventional SPE due to the lower usage of solvent and no conditioning of sorbent. Besides, d-SPE sorbents are not a single usage, unlike SPE sorbents. Thus, d-SPE techniques have been successfully performed as a clean-up method for vitamin B by using graphene oxide (GO) [9] and hydrophilic multi-template molecularly imprinted biopolymers (mt-MIBP) as novel adsorbents [10]. GO has hydrophilic and polar character due to the large quantities of oxygen atoms on its surface as carbonyl, hydroxyl group, epoxy, and polar group. These characteristics make it appropriate for the extraction of vitamin B_{12} [9].

Meanwhile, ultrasound-assisted dispersive solid-phase extraction (UA-DSPME) was used to extract vitamin B from juices. Based on a previous study, glucose was used to replace carbon sphere and deposit hydrophilic chitosan onto d-SPE sorbents for a green synthesis approach [10]. Satisfactory recoveries of 75.8%-92.7%, 83.3%-92.5%, and 84.7%-93.8% for B_2, B_3, and B_6, respectively, were obtained in this study [10]. Besides that, nanofibers have been used oftenly as adsorbents for packed-fibre SPE (PF-SPE) to extract three water-soluble vitamin B compounds from urine simultaneously with 84.9%–125.4% recovery range. Xie et al. in 2018 published a strategy employing polypyrrole nanofibers as the sorbent for PF-SPE. Diphenylboronic acid 2-aminoethyl ester (DPBA) was used as a complexing agent to enhance retention of three vitamins on the SPE column due to its multi-interaction with vitamins [11]. The main advantage of SPE is its stability of modification according to a compound of interest. However, the disadvantage of SPE in the vitamin analysis is low recoveries for highly polar vitamins on the C_{18} column [12].

Protein precipitation

Protein precipitation is one of the common methods used to release all the peptide-vitamin linkages. Protein precipitation was used for samples such as serum, urine, milk, and blood for extraction of water-soluble vitamins such as vitamin B. This is due to the samples being complex mixture compounds that biologically and actively bond to the analyte of interest, interfere, and have a negative impact on the stability of the analyte [4]. In a study on the determination of the native form of vitamin B_1 in bovine milk, different acids for the acidic protein precipitation were compared. Perchloric acid (7%), acetic acid (2.5 mol/L or 5.0 mol/L), hydrochloric acid (1.0 mol/L), and trichloroacetic acid (4%) were used for the preparation of the same commercial milk sample. The result showed that acetic acid at a pH level of 3.5 performed the best, resulting in 0.58 ± 0.03 µmol/L thiamine monophosphate (TMP) and 0.83 ± 0.03 µmol/L thiamine, yielding 0.43 ± 0.02 mg/L of total vitamin B_1 which represented as 100%. Meanwhile, for the protein precipitation of the milk sample, at pH levels of 4.5 and 5.0 proved to be insufficient because of high standard deviations. Furthermore, at all pH levels, hydrochloric acid and acetic acid performed significantly better than trichloroacetic acid and perchloric acid [13].

There are different solvents been used in the protein precipitation method for the determination of vitamin B in human milk. Study conducted by Ren *et al*. compared to the removal of protein by using methanol, acetonitrile with diethyl ether, and ethyl acetate were applied to remove the non-polar constituents and to assure the efficiency of the analysis [14]. The extraction recovery rates were 80.31%–118.63% for using either methanol or acetonitrile (ACN) for B_1, B_2, B_3, B_9, and B_6. However, the recovery percentage showed the best result by using methanol (80.7%-93.9%) compared to ACN (22.0%-66.7%) for the determination of pantothenic acid, nicotinic acid, pyridoxamine, and pyridoxal. Thus, methanol was selected in removing the protein. Furthermore, other step for pre-treatment was extracting the non-polar constituents and the response of using ethyl acetate was nearly twice lower than using diethyl ether [14].

Ultrasonic-assisted extraction

Ultrasonic-assisted extraction (UAE) is one of the methods that have high efficiency. Compared to soxhlet extraction and maceration, UAE has advantages not only in reducing the usage of solvent, but it also has high reproducibility and reduces the extraction time. This is due to the ultrasonic energy that is able to enhance the efficiency of extraction through induced cavitation. Moreover, this pre-treatment method produces high amount of yield and quality of an extract and fast mass transfer. Figure 1 shows the information block diagram of UAE process [4].

A previous study found that the efficiency of UAE of cyanocobalamin was greater than that of heat extraction [15]. Overall, the mean cyanocobalamin in the heat-treated ones with the added sugar alcohols was lower than in ultrasonic-treated samples (without and with added sugar alcohols). This was due to the formation of cavitation bubbles by ultrasound which caused mechanical and chemical effects. Thus, it led to the supplement's matrix broke down and cyanocobalamin was released. Furthermore, the appearance of very fine emulsions from immiscible liquid because of sonication and significant improvement in the interfacial contact areas between the liquids and a dramatic accelerate in the mass transfer among them. On the other hand, cavitation which appears following ultrasound exposure causes more available surface area and improves the procedure extraction efficiency, and also assists the release of interesting extractable compounds. Furthermore, efficiency can be increased based on the best selection of adsorbent and method [16].

The mechanical effects of ultrasound cause higher penetration of the solvent into cellular materials and also improve mass transfer. The ultrasound in the extraction can also disrupt the cell walls, helping the release of the content. There were a few studies where it gives the impact of pre-treatment steps such as drying and particle size in evaluating extraction efficiency. Mannaï *et al.* reported used instant controlled pressure drop (DIC) as a pre-treatment prior to ultrasonic extraction for the preservation of vitamin B_1 and B_6 [17]. It will helps to preserve heat-sensitive molecules because of short time during heating and lower the thermal degradation of the biological components. DIC is a process that has an effect of structure expansion to improve the technological aptitude of raw material which has been proved that the greater the expansion rate, the better the diffusivity constant [17].

Dispersive liquid–liquid microextraction (DLLME)

There is another miniaturised pre-treatment techniques of LLE which is known as dispersive liquid-liquid microextraction (DLLME). The significance of DLLME is that it is a powerful pre-treatment technique due to its simplicity, requires small amount of sample and low solvent consumption, has high enrichment factor, and short analysis time [12]. The DLLME method was compared with the traditional LLE methods in a study, results showed that the abundant contact surface of analytes and fine droplets increase the mass transferring processes of analytes from the aquatic phase to the organic phase in a DLLME process. Thus, it not only solved the time-consuming problem but also enhanced the extraction efficiency [4].

The new version of DLLME, which is known as ion pair-based dispersive liquid–liquid microextraction (IP-DLLME) has been used as a pre-concentration technique for five folate and B_9 derivatives from complex food matrix with enrichment factor ranged from 108 to 135 due to problem transfer of hydrophilic vitamins into the organic phase. Ethanol is usually used as the dispersion solvent meanwhile 1-octanol as the extraction solvent. In order to assist the transfer of hydrophilic folates into the organic phase in the extraction process due to the anionic structure of folates ions, quaternary ammonium salt (aliquot-336) is used as a cationic surfactant [18]. However, there are only a few applications of DLLME for the extraction of hydrophilic vitamins due to the use of chlorinated solvents as extraction solvents, which make these techniques less suitable for the extraction of vitamin B [12].

Summary of sample pre-treatment methods

Among all the pre-treatment methods, solid-phase extraction (SPE) is preferred for vitamin B analysis. SPE sequence with its online coupling with LC followed by various detectors show advantages of the less human handling which results in high sensitivity and accuracy. Moreover, multiple vitamin compounds can be quantified simultaneously in a single run by SPE; thus, a significant reduction in the sample preparation time. Therefore, the optimisation of the parameter is very important in a sample preparation. In order to confirm the optimised conditions, the fractional factorial design has been used in some studies to investigate the effect of the extraction conditions that are able to produce efficient extraction or clean-up of the target analytes from the sample matrix. The disadvantages of conventional SPE method used for extraction of vitamin B is relatively expensive because the SPE cartridges are intended for single-use, tedious, and require a long time and effort in method development. The pre-treatment methods used in previous studies are summarised in Table 2.

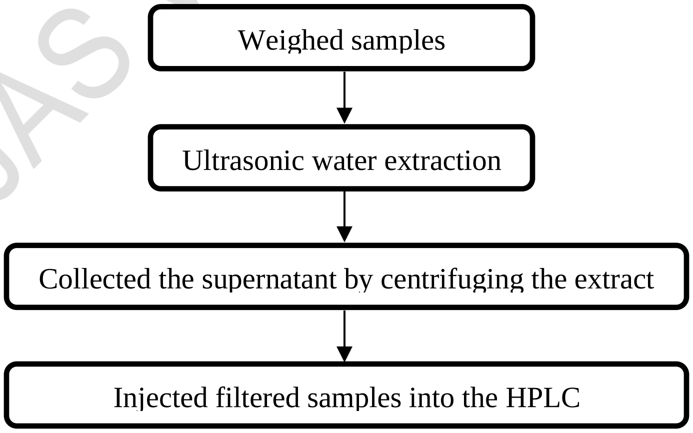


Figure 1. Ultrasonic-assisted extraction process [4]

Table 2. Pre-treatment methods, sample matrices and analytes of the recent articles

Pre-treatments	**Sample Matrices**	**Analytes**	**References**
Ultrasonic-assisted extraction (UAE)	Energy drinks.	Caffeine and water-soluble vitamins.	[19]
Hydrolysis	Fortified food and nutritional supplements.	Vitamin B_{12}.	[20]
Dilution	Pharmaceutical and biological fluids.	Thiamine hydrochloride, vitamin B_1.	[21]
UAE	Pharmaceuticals and urine samples.	Vitamin B_6, pyridoxine.	[22]
Dilution	Dietary supplements.	Vitamin B_{12}.	[23]
Enzymatic digestion, hydrolysis	Infant formulas	Vitamin B_1, B_2, B_3, and B_6.	[24]
Dilution (isotope)	Human plasma	Vitamin B_2.	[25]
Dilution	Oral powder for veterinary consumption.	Vitamin B_1, B_2, B_3, B_6, and C.	[26]
Centrifugation, filtration	Infant and toddler milk formulas.	Vitamin B_{12}.	[27]
Protein precipitation	Cow milk	Vitamin B_2 and B_6.	[28]
Enzymatic digestion	Cereal-based baby foods.	Vitamin B_1, B_2, and B_3.	[29]
Protein precipitation	Fresh milk from cow, goat, buffalo and pasteurised cow milk.	Vitamin B.	[3]
Enzymatic treatment	*Faba* bean seeds.	Vitamin B.	[30]
Ultrasound-assisted dispersive solid-phase microextraction (UA-DSPME)	Human urine and milk samples.	Nicotinamide.	[31]
Immunoaffinity	Mealworm, cricket, grasshopper, cockroach.	Vitamin B_{12}.	[32]

Table 2 (cont'd). Pre-treatment methods, sample matrices and analytes of the recent articles

Pre-treatments	**Sample Matrices**	**Analytes**	**References**
SPE (Graphene oxide)	Pharmaceutical formulations.	Vitamin B_{12}.	[9]
Dilution	Pharmaceutical products and urine sample.	Antidepressant drugs and vitamin B_{12}.	[33]
Acid hydrolysis, enzymatic digestion	Bovine milk.	Vitamin B_1.	[13]
Protein precipitation and enzymatic treatment	Breast milk.	B-vitamers.	[34]
SPE (polypyrrole as sorbent)	Urine sample.	Vitamin B.	[11]
Protein precipitation	Human milk	Vitamin B.	[14]
UAE (DIC)	*Steviarebaudiana* bertoni	Vitamin B_1 and B_6	[17]
Ion pair based dispersive liquid–liquid microextraction (IP-DLLME)	Food matrix	Vitamin B_9	[18]

Analysis methods

Although a few studies have used HPLC and mas spectrometry (MS) detectors due to their high specificity and sensitivity, UV, PDA, DAD detections are still being used as they are less expensive. Based on the chemical structure of vitamin B compounds, these compounds absorb at approximately 256 nm. There are many different methods such as electrophoretic, biosensor, and microbiological assay. The development of such methods; sensors and biosensors for food freshness detection has attracted many researchers, especially in the food sector. Despite the achievement obtained so far, there are still some improvements needed.

Liquid chromatography

Liquid chromatography (LC) has been widely used due to its high sensitivity and broad linear range. Nowadays, reversed-phase high performance liquid chromatography (RP-HPLC) has been used in analytical methods to determine water-soluble vitamins such as vitamin B. This method with the combination of a polar mobile phase and non-polar stationary phase becomes popular due to some advantages over normal phase such as better peak shape, its compatibility with MS detection, the robustness of chromatographic columns, and also fast equilibrium. Furthermore, there are several types of stationary phase that have been used in RP-HPLC but the one that being used widely is C_{18} for the separation of vitamin B by eluting with a mixture of water and organic solvents such as methanol, acetonitrile and ethanol and also small percentage of acids such as trichloroacetic acid, hexane sulfuric acid, and formic acid [3, 10, 25, 28, 35, 36, 37]. Simultaneous extractions of different analytes of vitamin B such as B_1, B_2, nicotinic acid, nicotinamide, B_5, and B_{12} was successfully done by using the C_{18} column as a stationary phase [10, 24, 33, 38, 39].

LC coupled with MS and multiclass analyses

HPLC–MS/MS has become the main analytical technique used for the determination of vitamin B due to its higher selectivity and sensitivity compared to other instrumental methods. Furthermore, the basic principle of this method is the measurement of the mass-to-charge (m/z) ratios of ionised molecules, and it is used to quantify and identify a substance where it can confirm a compound's molecular structure. Usually, this detection technique is applied by using a triple quadrupole analyser and a selected reaction monitoring mode, which allows the confirmation of the composition of compounds and gives structural information. Besides, the advantage of MS/MS is the fact that a complete HPLC separation of the target analytes is not necessary for selective detection. Instead, it is advisable to have a good chromatographic separation to reduce matrix effects which result in the suppression or less frequently in the enhancement of analyte signals. Thus, to consider speeding up the analysis, short HPLC columns are often used [4].

The method was established to determine simultaneously total vitamins B_1, B_2, B_3, and B_6 in infant formula and related nutritional foods. This method uses acid phosphatase to dephosphorylate the phosphorylated vitamin forms and then measures thiamine, riboflavin, nicotinamide, nicotinic acid, pyridoxine, pyridoxal, and pyridoxamine from digested sample extract by LC-tandem MS. In addition, the presence of modern MS instrumentation with electrospray ionisation (ESI) helps simultaneous determination by removing remaining hurdles associated with the detection. In this study, prepared samples and standard solutions were injected onto an ultra-high pressure liquid chromatography (UHPLC) which then interfaced to a triple-quadrupole mass spectrometer for analysis, where the function is to monitor parent-daughter (precursor-fragment) ion pairs for each analyte and internal standard. Intermediate precision average was 3.9% and over-spike recovery was generally 95%-105% for all four vitamins [24].

LC–MS/MS method was established for the determination of nicotinamide, nicotinic acid, B_1, B_6, B_2, B_5, B_7, B_9, and B_{12} in fish. Baseline separation of the vitamins was achieved in a hydrophilic interaction of LC condition. LOQ values ranged from 0.4 to 50 ng/g for the different vitamins while the spike recovery ranged from 87.5 to 97.5% at values 50 and 100 ng/g [40].

Liquid chromatography coupled with other techniques

Usually, RP-HPLC will be used with ultraviolet (UV), fluorescence (FLD), or photodiode array (DAD) in process of quantification of vitamins in different types of samples. However, they are not able to provide direct evidence of the structure or composition of a substance even if these approaches are quantitative. UV detection has the least selective and sensitive, but it is the most affordable and versatile method. Meanwhile, FLD is found to be more selective and sensitive compared to UV.

The separation and quantification of ten B vitamins in pretreated human milk samples have been successfully performed within 11 minutes by using the UPLC–MS/MS method with an Acquity UPLC HSS T3 column (2.1 × 100 mm, 1.8 µm). The homogenised human milk samples were deproteinised by using methanol and unknown contaminants were extracted using diethyl ether then, the hydrophobic phase was discarded. The analytes were monitored by using ESI+ ionisation and with three acquisition functions, it was detected in multiple reaction monitoring (MRM) [14].

A previous study established an isotope dilution method for determination of riboflavin in human plasma by using LC–MS/MS where separation performance was obtained with an Agilent 1290 UHPLC combine with Poroshell 120 SB-Aq column (100 mm × 2.1 mm × 2.7 µm) and was operated in positive ion mode. This method required a short execution time (2.5 min) and the average recovery was 90.5%–99.5%. Thus, it is reliable to be applied in clinical diagnosis of deficiency and supplementation monitor of riboflavin [25].

A simple and rapid method for determination of thiamine in red wine by using HPLC coupled to fluorescence detector was conducted.

Polyvinylpolypyrrolidone was used to remove phenolic compounds from red wines and allow a spike recovery of thiamine vitamers (101% for thiamine, 104% for TMP, and 100% for TDP) in a wide range of red wines [39]. Unfortunately, this method encountered a problem where the interaction of polyphenols with ferricyanide had caused the effectiveness of ferricyanide oxidation in the derivation process to decrease. Thus, polyvinylpolypyrrolidone was used to remove polyphenols so that it allows the accurate determination of thiamin vitamers by calculating its mean of HPLC with fluorescence detection (HPLC-FLD).

Electrophoretic methods

Capillary electrophoresis (CE) is also a good quantitative analytical method that usually used when there are only small amounts of a sample available. It has several advantages such as highly efficient, low solvent consumption, and also fast techniques where the sample components are separated according to their sizes and charges. Furthermore, this method also ables to analyse several samples simultaneously in multicapillary systems and consume low volume of reagents and accessories (packaged columns are not required). However, chromatographic techniques are more sensitive for the determination of vitamin B compared to CE methods, even though it has greater efficiency when compared to LC methods.

Mostly the analysis of vitamins used the CE method by employing capillary zone electrophoresis (CZE) or micellar electrokinetic chromatography (MEKC) modes where they are coupled with laser-induced fluorescence (LIF), UV, electrochemical (EC), and MS detectors. Vitamin B is in the category of water-soluble vitamins and as charged molecules, it can be separated by using CZE whereby their separation efficiency can be improved by applying MEKC to simultaneously separate fat-soluble and water-soluble vitamins [41].

In MEKC, when micelles of surfactants in the background electrolyte (BGE) acts as a pseudo-stationary phase, electrostatic and hydrophobic interactions occur with analytes. A MEKC method was established in a study to analyse eight water-soluble vitamins by using HP3D CE system with DAD in energy and sports drinks. Sodium dodecyl sulphate (SDS) gave the best separation in this study because it was able to resolve the B_5–C vitamin pair as compared to other tested surfactants including sodium deoxy-cholate (SDC) and sodium cholate (SC). However, this technique was not suitable for the determination of vitamin B_{12} due to the very low concentration below the LOQ [42].

Biosensors

Optical biosensors is one of the excellent tools to detect vitamins in different matrices due to their advantages which are simplicity, the possibility to be used in field analyses, and low cost. For example, electrochemical sensors that are based on the use of receptors fabricated through different imprinting approaches have been developed for the detection of vitamin B [22, 43-46]. Furthermore, electrochemical techniques have shown interest in the recent research development because of their advantages such as high sensitivity, easy operation, low cost, and also the portability of the equipment used.

A study proposed an electrochemical method for the determination of vitamin B_{12} where the Co(I/II) redox pair was monitored by using a boron-doped diamond electrode for the analysis of supplementation products, where its surface was characterised by electrochemical impedance spectroscopy and cyclic voltammetry. The results were compared with those provided by UV-Vis spectrometry and the statistical analysis showed no critical difference between these two methods in terms of the precision and accuracy of the data. The electrode was sensitive to vitamin B_{12} sensing in the linear range from 0.25 to 5.0 μmol^{-1} with a value of LOD of 86.0 $nmolL^{-1}$ [44].

Meanwhile, another study had established the electrocatalytic method for the determination of vitamin B_6 by using vanadium (III) Schiff base complex that was electrochemically polymerised using glassy carbon electrode (GCE) as the working electrode. This method gives a faster response, cost-effective, good accuracy, and simple procedure. They utilised vanadium (III) Schiff base complex for the

GCE active surface modification. Furthermore, vanadium complexes show a good electrochemical redox behaviour because of their different oxidation states (+3, +4, +5). Thus, poly-V-SBC/GCE shows better electrocatalytic sensing activity for the determination of vitamin B_6 [46].

Other methods

On the other hand, there is also an effective method such as a microbiological assay that has been developed to determine vitamin B. This method is justified when it needs to carry out quality control routine of relatively simple sample compositions. However, this method is challenging when applied to newer, more diversed nutritional products and is no longer considered the gold standard. Newer methods such as chromatographic methods, especially with mass spectral detection, becomes the new standard due to their specificity that enable accurate quantitation across more complex and diverse matrixes.

Summary of analysis methods

Among all of the analytical methods, LC is the most popular due to its advantages. LC methods can meet the requirements of the qualitative and quantitative analyses of vitamins in different matrices, such as foods and biological samples, especially when they are combined with MS. However, UV and FLD detectors suffer overlapping peaks when dealing with complex samples, while matrix effects and high costs are necessary when using LC–MS. CE is an alternative method for the determination of vitamins which is highly efficient, low solvent-consuming, and fast, but its separation reproducibility needs to be enhanced. The main advantages of biosensors include their low cost, technical simplicity, and the possibility of being used in field analyses, while their relatively short lifespan restrict the development of technology. Spectrometry is cheap and easy to promote, but its sensitivity does not quite meet requirements sometimes. In conclusion, with the development of equipment of HPLC and MS, this technology will surely be broadly used, while other technologies, such as electrophoresis and spectrometry, are seen as supplementary methods to be used when necessary. Table 3 presents a selection of analytical methods used for the detection of vitamin B reported since 2015.

Table 3. Summary of separation, detection, and quantification methods used for the analysis of vitamin B

Vitamin B forms	Mobile phase	Column	Instrument analysis method	LOD	LOQ	Ref
Vitamins B_1, B_2, B_3, B_6, B_9, B_{12}, and C	A: 5.84 mM hexane-1-sulfonic acid sodium: acetonitrile (95:5) with 0.1 % triethylamine B: 5.84 mM hexane-1-sulfonic acid sodium: acetonitrile (50:50) with 0.1 % triethylamine	C_{18} BDS column (10 cm × 4.6 mm; 3 µm)	HPLC with UV-DAD/FLD detection	0.0032–0.0267 mg/L	0.0097 – 0.0810 mg/L	[38]
Vitamins B_1, B_2, B_3, B_5, B_6, B_9, B_{12}, and C	A: 0.01% formic acid B: methanol	Phenomenex Synergi, C_{18} (4.6 × 150 mm; 4.0 µm particles	HPLC	0.03 – 0.23µg/mL	0.18 – 0.67 µg/mL	[47]

Table 3 (cont'd). Summary of separation, detection, and quantification methods used for the analysis of vitamin B

Vitamin B forms	Mobile phase	Column	Instrument analysis method	LOD	LOQ	Ref
Vitamins B_2, B_3, and B_6.	Water:methanol:acetic acid (72%:27 %:1%, v/v)	Agilent C_{18} column (4.6 × 250 mm; 5 µm)	HPLC	1.2 – 5.5 µg/L	4.0 – 18.4 µg/L	[10]
Vitamin B_{12}	A: 20 mM ammonium formate in H_2O B: Acetonitrile (ACN)	:UG120V C_{18} (1.5 × 250 mm; 5 µm)	LC–MS/MS	0.03 µg/L	0.1 µg/L	[27]
B-vitamins	A: 2.5mmol/L Ammonium formate aqueous solution. B: ACN	Acquity UPLC HSS T3 (2.1 × 100 mm; 1.8 µm)	UPLC–MS/MS	0.05 – 1.50 µg/L	0.25 – 3 µg/L	[14]
B-vitamins	A: 10 mM ammonium acetate, 0.1% acetic acid in water B: methanol	Agilent Zorbax SB-Aq column (100 × 2.1 mm; 1.8 µm particle size), SB-Aq guard column (5 × 2.1 mm; 1.8 µm)	UPLC–SRM MS	0.01 – 0.625 µM	0.1 – 26.3 µM	[30]
Vitamin B_1 and B_6	A: 25 mmol/L potassium phosphate buffer pH 7 B: Methanol	YMC meteoric core C_{18} column (100 × 4.6 mm; 2.6 µm particle size) protected by guard column C_{18} (10 × 4.6 mm)	HPLC-PDA and fluorescence	1.28 – 2.50 nmol/L	4.27 – 8.32 nmol/L	[48]
Vitamin B_{12}	A: 20 mmol/L potassium phosphate monobasic (adjusted to pH 3 with phosphoric acid) B: ACN	Express C_8 column (2.7 µm; 3 × 100 mm), guard column (2.7 mm; 3 × 5 mm)	HPLC-DAD	0.16 µg/mL	0.52 µg/mL	[23]
Water-soluble vitamins	A: 25 mM $NaH_2PO_4.H_2O$, pH adjusted to 4.0 B: Methanol	XSelect ESH C_{18} column (150 × 4.6 mm; 3.5 µm particle size)	UHPLC-DAD	0.02 – 1.7 mg/L	0.05 – 5.1 mg/L	[49]

Table 3 (cont'd). Summary of separation, detection, and quantification methods used for the analysis of vitamin B

Vitamin B forms	**Mobile phase**	**Column**	**Instrument analysis method**	**LOD**	**LOQ**	**Ref**
Vitamin B_{12}	A: UHQ water modified with 0.025% trifluoroacetic acid B: Methanol	Acquity UPLC column (BEH C_{18}, 50 × 2.1 mm)	UHPLC	0.12 – 1.61 nM	0.40 – 5.34 nM	[32]

Conclusion

The development of analytical methods for the accurate determination of vitamin B and its metabolites is an important area in analytical chemistry because of the importance of these vitamins in the human body. In this review, the methods published during the last five years (2015–2020) were evaluated for vitamin B determination. The key roles of these methods are played by sample preparation techniques, and the main efforts in this field have been focused on the optimisation of the preparation, extraction, and clean-up steps and on the enhancement of the environmental safety of these procedures. Based on current trends, the method with the most promise in achieving these goals is the SPE sequence with its online coupling with LC followed by various detectors. The main advantages of this approach are its good compatibility with high throughput multi-residue analytical procedures and its relatively low cost.

The currently proposed analytical approaches for the detection of vitamin B are mainly based on HPLC–MS or HPLC–MS/MS. Great advances in HPLC–MS/MS have made it a key technique for the determination of not only vitamins but also other targets. The main trend in this field is the combination of MS detectors with modern chromatographic approaches such as UHPLC. Therefore, this technique is expected to have the most pronounced development in the future.

Furthermore, the development of new sample preparation methods using various novel materials, green solvents and miniaturised devices for improving the sensitivity, robustness and effectiveness of analytical methods, and the method standardisation should also be emphasised in the future.

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