Proceedings of the 2nd International Conference on Environmental Geoscience and Earth Ecology
DOI: 10.54254/2753-8818/37/20240183

Research progress on the detection of water-soluble vitamins in food using liquid chromatography

Jing Zhang[1,4], Hongye Zhang[2, 3, 5]

[1]China Medical University – The Queen's University of Belfast Joint College, China Medical University, Shenyang, 110122, China
[2]College of pharmacy, China Medical University, Shenyang, 110122, China
[3]Corresponding author

[4] zhangjing071173@163.com
[5]zhanghongye52@163.com

Abstract. Currently, liquid chromatography is widely used for the analysis and determination of water-soluble vitamins (WSVs) in functional foods. However, due to the diversity of sample characteristics and the requirement for purification accuracy, the chromatographic conditions and specific methods used vary. This paper reviews the chromatographic conditions of liquid chromatography techniques for detecting WSVs in food, as well as the selection of liquid chromatography methods in different application fields. The chromatographic conditions include chromatographic columns, column temperature, mobile phase, flow rate, and elution gradient, and the application scope of different chromatographic conditions is compared. Liquid chromatography methods include high-performance liquid chromatography and liquid chromatography-tandem mass spectrometry, and discussions are made on the applications and prospects of these two methods under different chromatographic conditions. Therefore, this review provides research insights into different chromatographic conditions and methods for food quality inspection and biochemical analysis research.

Keywords: Liquid Chromatography, Chromatographic Conditions, Water-Soluble Vitamins, Food

1. Introduction

Vitamins are a class of trace organic substances essential for maintaining normal physiological functions and metabolism in the human body, which can be classified into fat-soluble vitamins and water-soluble vitamins based on solubility [1]. Water-soluble vitamins (WSVs) include B-complex vitamins and Vitamin C (VC) [2]. Among them, B-complex vitamins exert influence on substance metabolism in the body by forming coenzymes [3], while VC serves as a coenzyme for hydrolytic enzymes involved in collagen and catecholamine synthesis as well as gene expression regulation [4]. Abnormal levels of these vitamins can lead to physiological and psychological health issues such as skin diseases, gastrointestinal disorders, endocrine disorders, mental illnesses, and cancers (see Table 1 for classification and functions of WSVs). Therefore, the aforementioned WSVs play significant roles in growth, metabolism, and development. Due to the body's inability to synthesize and/or store a sufficient amount of WSVs, their content in the body is minimal [5]. Even with a balanced diet providing most of the required vitamins,

DOI: 10.54254/2753-8818/37/20240183

there is still a risk of low intake of trace nutrients for most people [6], necessitating supplementation from functional foods containing specific doses of vitamins.

Table 1. Classification and functions of water-soluble vitamins

WSVs	Functions	Abnormal influence	Reference
Vitamin B_1 (VB_1)	Involved in energy production and central metabolism pathways	Deficiency leads to neurological disorders and beriberi	[6]
Vitamin B_2 (VB_2)	Prevents cancer, anemia, and migraines	Deficiency causes symptoms such as glossitis, angular stomatitis, and skin lesions; Excessive intake may lead to tissue and DNA oxidative damage and sporadic colorectal cancer	[7]
Vitamin B_3 (VB_3)	Participates in lipid metabolism, tissue respiration, and anaerobic breakdown of carbohydrates	Insufficient intake can lead to mitochondrial dysfunction, pellagra, gastrointestinal disorders, hypercholesterolemia, depression, etc.	[6, 8, 9]
Vitamin B_5 (VB_5)	Responsible for biochemical and signaling reactions within the body	Deficiency damages the skin, mucous membranes, and nervous system	[10]
Vitamin B_6 (VB_6)	Regulates basic cellular metabolism and influences overall physiological conditions	Deficiency causes symptoms such as loss of appetite, vomiting, depression, etc.; Long-term high-dose supplementation of pyridoxine can lead to sensory neuropathy	[9, 10]
Vitamin B_7 (VB_7)	Involved in fatty acid synthesis, gluconeogenesis, and branched-chain amino acid catabolism	Deficiency can lead to seborrheic dermatitis, loss of appetite, fatigue, etc.	[11, 12]
Vitamin B_9 (VB_9)	Growth and reproduction of organism cells	Insufficient levels can lead to DNA hypomethylation; Overexpression can cause cancer, anemia, and cognitive impairments	[6, 9]
Vitamin B_{12} (VB_{12})	Possesses anti-inflammatory and antioxidant properties, involved in red blood cell formation, synthesis and maintenance of myelin sheath, as well as synthesis of nucleic acids and neurotransmitters	Deficiency manifests as nonspecific symptoms, disrupts homocysteine balance	[9, 11, 13]
Vitamin C (VC)	Antioxidant, anti-inflammatory, antithrombotic, and immune-regulating properties, prevents common colds and cancer	Deficiency can lead to kidney disorders and even scurvy; Excessive supplementation disrupts physiological redox balance and cellular homeostasis	[4, 9]

Functional foods refer to foods with specific nutritional and health functions or intended to supplement vitamins and minerals, including health foods [14], infant formula [15-17], functional beverages [18], vitamin supplements [1-3, 5], nutrient fortifiers [16, 19-21], etc. These foods can improve the physiological functions of different populations and have been proven to reduce the risk of chronic diseases [22]. With the emergence of various functional foods in the market, on one hand, channels for people to obtain trace nutrients have expanded, while on the other hand, the problem of imbalance in nutrient doses has arisen. In order to better provide consumers with targeted functional foods, researchers have begun to focus on the purification and detection of water-soluble vitamins (WSVs) in food.

With the increasing demand for accurate determination of WSV content in various functional foods, various analytical techniques have been introduced for the purification and analysis of specific vitamins [23]. When the content of target compounds such as vitamins is complex, it is sometimes necessary to use multiple techniques to analyze the active ingredients of these vitamins. Traditional detection methods include microbiological methods [24], electrochemical methods [25], capillary electrophoresis

DOI: 10.54254/2753-8818/37/20240183

[26], thin-layer chromatography [27], etc. However, traditional methods cannot meet the requirements of modern detection for simultaneous detection of multiple vitamins. Compared to traditional methods, liquid chromatography offers better detection speed, reliability of results, optimal separation potential, and selectivity [17]. This paper focuses on the chromatographic conditions of liquid chromatography and the application of different liquid chromatography methods. Chromatographic conditions include chromatographic columns, column temperature, mobile phase, flow rate, and elution gradient. Liquid chromatography methods mainly include high-performance liquid chromatography (HPLC) [28-37] and liquid chromatography-tandem mass spectrometry (LC-MS/MS) [38-47]. HPLC has been widely used for the determination of WSVs due to its high sensitivity and low cost [40]. LC-MS/MS is widely used in clinical experiments due to its high stability, good specificity, and high sensitivity [40, 48]. When detecting WSVs in various foods, the analysis results vary due to different chromatographic conditions and methods [43].

In order to extract WSVs from functional foods or determine their content, researchers need to select appropriate chromatographic conditions and methods based on the properties of the analytes when using liquid chromatography. This review aims to provide a comparison of widely applicable chromatographic conditions and different liquid chromatography methods and their application fields, providing new insights for the selection of analytical methods in the production of targeted supplements and food quality inspection.

2. Impact of Chromatographic Conditions on the Detection of Water-Soluble Vitamins

Depending on the physicochemical properties of different analytes and the utilization of various chromatographic conditions and sample pretreatment techniques, substances with very similar properties can be simultaneously separated and determined to achieve the separation of trace components in complex mixtures [49]. Among them, the selection of chromatographic conditions is particularly important, and only by choosing appropriate chromatographic columns, column temperatures, mobile phases, flow rates, and elution gradients [19], can good separation results be achieved.

2.1. Chromatographic Columns and Column Temperature

In liquid chromatography, chromatographic columns are usually made of polished stainless steel or heavy glass materials and filled with stationary phases. Analytical columns are mainly used for quantitative analysis of samples [50], with C18 chromatographic columns [51] being the most common packing material. R.B.H. Wills et al. [4] compared μ Bondapak C_{18} and μ Bondapak NH2 columns for the simultaneous separation of seven WSVs from a mixture in 1977. The results showed that each vitamin could be eluted well from the μ Bondapak C_{18} column, with an analysis time of less than 40 minutes, while the μ Bondapak NH2 column, although shorter in analysis time by 15 minutes compared to the C_{18} column, could not elute folic acid and had lower sensitivity than the C_{18} column. Shi-Qi Zhang et al. [18] prepared layered porous submicron flow-through silica microspheres (Sub-FTSiO2) with particle sizes ranging from 2.5 to 3.5 μm by combining suspension polymerization with sol-gel transition and phase separation methods. They conducted experiments using a hydrophilic interaction liquid chromatography (HILIC) column, where a large number of penetrable mesopores provided good separation capability and permeability, achieving rapid separation of seven WSVs in functional beverages within 2.2 minutes. Although this method enables rapid separation and determination of WSVs, it requires additional preparation of packing materials, involves multiple pretreatment processes, and is relatively complex, resulting in higher analysis costs compared to C_{18} columns.

From the above analysis, it is evident that the C_{18} column exhibits better applicability in research. Ho Jin Kim [28] conducted experiments detecting six WSVs with detection limits (LOD) ranging from 25 to 197 μg/kg and quantification limits (LOQ) ranging from 84 to 658 μg/kg. Marco Ciulu et al. [33] detected WSVs in honey with LODs ranging from 0.10 to 0.58 mg/kg and LOQs ranging from 0.3 to 1.75 mg/kg. Chunmei Geng et al. [35] detected four WSVs, with accuracy and precision both exceeding 15%. These methods collectively demonstrate the high sensitivity and wide applicability of the C_{18}

DOI: 10.54254/2753-8818/37/20240183

column. For different chromatographic columns, the combination-separation of samples and stationary phases may vary due to different column temperatures, leading to differences in peak areas and retention times of each WSV, and consequently, different chromatographic analysis results.

Taking the C_{18} column as an example, at room temperature (25°C) [29], the HPLC separation of five WSVs is satisfactory, reaching baseline separation within 22 minutes. At 30°C [48], the tailing factors of seven WSVs are all less than 1.5, with relatively good peak shapes for each component. At 40°C [28], the average sample recovery rate of six WSVs ranges from 82.3% to 98.9%, reflecting the reliability of the analytical method. Therefore, when the column temperature is between 25°C and 40°C, it ensures both the retention time and separation time of the samples in the column, as well as good chromatographic resolution. In summary, when detecting WSVs in functional foods, the use of C_{18} columns is more cost-effective, and temperatures ranging from 25°C to 40°C are more suitable for separating vitamins.

2.2. Mobile Phase, Flow Rate, and Elution Gradient

When detecting WSVs, the mobile phase generally consists of buffer solutions or formic acid aqueous solution-methanol or acetonitrile systems [28-37, 42, 46, 47]. Under certain experimental conditions [31], WSVs in jujube coconut were detected using a buffer solution-methanol (96:4) system with pH adjusted to 3.0 with orthophosphoric acid. The chromatogram produced a sharp and compact peak with a recovery rate of 99.05% to 100.11%. The intra- and inter-group variations at two different concentration levels showed relatively low relative standard deviations (0.90%-1.02%), demonstrating the high reproducibility and accuracy of the system. Based on the close polarity of the analyzed vitamins, namely WSVs, defined in literature [3], the necessity of using buffered mobile phases was established. Experimentation with methanol and acetonitrile as organic modifiers found that their mixture exhibited the best selectivity. Ultimately, a phosphate buffer (pH=4): acetonitrile (98:2) and methanol: water (50:50) system was used for gradient elution at a flow rate of 0.8 mL/min. The gradient method applied ensured good separation of all WSV components in the mixture.

Careful selection of the mobile phase is necessary as improper use of mobile phase reagents may lead to co-elution, baseline and time drift, complex backgrounds, etc. [3], and thus, it cannot be generalized. Due to the strong polarity and ionization capacity of WSVs such as VB_1, VB_2, VB_6, VC, their retention capacity on C_{18} columns is often poor and may require the addition of ion-pairing reagents [14, 28] to prolong the retention time of analytes, increase separation, and improve chromatographic peaks. For instance, Chen Caiyun et al. [14] found that the components of health foods such as VB_1, VB_2, VB_5, VB_6, niacin were difficult to separate in acetonitrile-water and methanol-water systems, but separation efficiency improved with the use of acetonitrile-ion-pair reagent system (5 mmol/L sodium heptanesulfonate), with separation efficiency of the five vitamins >1.5. Additionally, Ho Jin Kim's study [28] used buffer A (5 mM PICB-6, 0.1% CH3COOH) and buffer B (5 mM PICB-6, 65% methanol) as the mobile phase to analyze six WSVs in animal feed and optimized the extraction process using PICB-6 as the ion-pairing reagent. The LOD was 25-197 μg/kg, LOQ was 84-658 μg/kg, and the average sample recovery rate of the six WSVs was 82.3%-98.9%. The intra- and inter-day variations in peak area changes were less than 5.6%, demonstrating the reliability of the method. Although higher concentrations of ion-pairing reagents result in longer retention times for samples, they also cause greater damage to the chromatographic column, so the concentration of ion-pairing reagents needs to be properly controlled [14]. In addition to the mobile phase, flow rate and elution gradient also affect the separation efficiency and chromatographic behavior of vitamins.

Most researchers [28, 29, 31-33, 37, 42, 46, 47] choose gradient elution for the separation of WSVs at flow rates between 0.2-1 mL/min. Xiaoqin Cheng et al. [30] employed a gradient elution with 50 mmol/L ammonium carbonate-methanol at a flow rate of 0.25 mL/min, resulting in elution times of less than 3.23 min for four types of VB_1. C. Kadakal et al. [29] utilized a gradient elution with 50 mM KH_2PO_4 (pH 7)-100% methanol at a flow rate of 1 mL/min, separating five WSVs in bulgur samples within 22 min. Labaran IBRAHIM et al. [34] achieved isocratic elution using methanol-phosphoric acid (35:65) at a flow rate of 1 mL/min, with B_{12} retention time in fish being less than 5.0 min. Yi Yang et al.

DOI: 10.54254/2753-8818/37/20240183

[8] observed that as the flow rate increased, chromatographic pressure gradually increased, resulting in decreased retention times and peak areas for vitamins, while too low flow rates led to excessively long retention times and low pressure. It can be seen that different flow rates affect the elution of WSVs from samples, and appropriate retention times require suitable flow rates to achieve good baseline separation between target peaks and interfering peaks in actual sample detection, thereby improving analysis efficiency. The choice of elution gradient is based on the complexity of the sample. WSVs in actual samples typically exhibit large differences in retention, and HPLC under constant elution conditions (isocratic mode) may not achieve sufficient separation [52]. Therefore, gradient elution, which changes the polarity of the mobile phase by altering the composition of solvents, allowing for the optimal separation of different vitamins in the shortest time, is adopted to enhance column efficiency. Thus, when detecting WSVs, the mobile phase may use a buffer solution-methanol/acetonitrile system, but comprehensive considerations based on the characteristics of the sample itself are necessary for selection and optimization. When the analytes are complex, containing multiple different WSVs, appropriate flow rates for gradient elution can be chosen based on the retention times of the samples; when the analytes are simpler, containing only 1 to 3 WSVs, isocratic elution can be conducted at flow rates between 0.2-1 mL/min.

In conclusion, different chromatographic conditions lead to varying results in the determination of WSVs in functional foods. Setting the chromatographic conditions as a C_{18} column, a column temperature of 25-40°C, and a mobile phase consisting of a buffer solution-methanol/acetonitrile system with gradient elution demonstrates general applicability. However, it's important to note that further experimentation and optimization of the mobile phase should be conducted based on the properties of the measured samples and the characteristics of WSVs. Additionally, the selection of flow rate should be based on the capacity of the chromatographic column and the retention times of WSVs. Setting the chromatographic conditions is a prerequisite for separating vitamins in food, and choosing the appropriate liquid chromatography method is fundamental to the extraction and detection of WSVs.

3. Application and Comparison of Different Liquid Chromatography Methods

Liquid chromatography is a separation and analysis technique where the mobile phase is liquid and the stationary phase is solid or liquid. This technique includes HPLC and LC-MS/MS, with HPLC further divided into reverse-phase liquid chromatography (RP-HPLC) and hydrophilic interaction liquid chromatography (HILIC) based on the different chromatographic columns. Below, we will introduce and analyze these three methods.

3.1. Reverse-Phase Liquid Chromatography (RP-HPLC)

HPLC separation modes include normal phase (NP) and reverse phase (RP) [50]. In NP-HPLC mode, the stationary phase is polar while the mobile phase is non-polar, used for rapid elution of non-polar substances. In RP-HPLC mode, the stationary phase is non-polar while the mobile phase is polar, used for rapid elution of polar substances. Most WSVs are highly polar with varying degrees of hydrophilicity, making them more easily separated and eluted first using RP-HPLC mode, which also offers higher selectivity [39] and is more advantageous in terms of time and cost compared to normal phase mode. Due to its simplicity and efficiency [36], most researchers utilize RP-HPLC for the separation and determination of WSVs [1, 28-35, 37].

Based on the Applicability of RP-HPLC, various researchers have utilized this method for the separation and analysis of WSVs in functional foods. R.B.H. Wills et al. [4] achieved optimal resolution of seven vitamins by adding PIC reagent (pH 7.0) in a 70:30 water-methanol solvent system using UV detector. However, this method requires separate detection of interference in each type of food and the development of techniques to remove interference, limiting its application to a certain range of foods. Niu Canjie et al. [32] selected the method of using a diode array detector (DAD) coupled with a fluorescence detector (FLD) to detect VB_1, VB_2, niacin, niacinamide, and VB_6 in nutrient-fortified starch. The LOD ranged from 0.01 to 0.08 mg/100g, LOQ ranged from 0.025 to 0.26 mg/100g, with spike recovery rates of 90.5% - 102.5% and relative standard deviations of 0.67% - 3.45%, demonstrating

DOI: 10.54254/2753-8818/37/20240183

high precision and recovery rates. This method can enhance detection sensitivity, reduce detection limits, and minimize matrix interference and interference peaks' impact. However, the types and quantities of samples studied are relatively limited. Alexandrina Mateeva et al. [3] used RP-HPLC with DAD to test various reverse-phase columns and found that the Purospher STAR C_{18} (Merck Millipore) 5 μm, 25 × 0.46 cm column was most suitable for retaining and separating water-soluble and fat-soluble vitamin mixtures. The LOD ranged from 0.03 to 9.23 μg/mL, LOQ ranged from 0.08 to 27.30 μg/mL, with average recovery rates between 97.6% and 101.0%, indicating good sensitivity and accuracy. They subsequently performed quantitative analysis on seven food supplements and one secondary fermented beer yeast, obtaining results consistent with the labeled contents, confirming the method's applicability for routine analysis. Therefore, RP-HPLC has become increasingly refined over time, with characteristics of high sensitivity, selectivity, and reproducibility, making it suitable for the analysis and detection of WSVs in most foods, as well as in industrial food sectors and environmental samples [34, 37].

However, chromatographic separation of WSVs using standard RP-HPLC poses certain challenges [39] due to their high polarity, resulting in poor retention times and chromatographic behaviors. Although altering the mobile phase and analysis conditions can improve this, it may still not achieve the desired results. HILIC is an alternative method to RP-HPLC.

3.2. Hydrophilic Interaction Liquid Chromatography (HILIC)

The separation mechanism of HILIC is opposite to that of RP-HPLC, utilizing hydrophilic interactions between the stationary phase and the mobile phase, making it very suitable for separating polar substances on polar stationary phases using organic-rich aqueous mobile phases [2]. Swen Langer and John K. Lodge [19] first used HILIC to simultaneously detect multiple WSVs extracted from complex food matrices, employing a gradient elution of 10 mM aqueous ammonium acetate (pH 5.0): acetonitrile (95:5), enabling the simultaneous detection of six B-group vitamins within 18 minutes, with intra-day and inter-day repeatability of 1.6% - 3.6% and 1.8% - 11.1%, respectively. Due to the excellent solubility of bioactive compounds in the HILIC environment, the application of HILIC in the separation of WSV mixtures has been a strong trend in recent years [36]. Huiliang Geng et al. [53] manipulated the carbon load of the stationary phase by preparing a novel HILIC polar stationary phase, HPG-Sil, through surface-initiated polymerization of hyperbranched polyglycerol with silica. This stationary phase exhibited significant hydrogen bonding interactions and typical HILIC characteristics, demonstrating a new universal separation ability for polar analytes. For example, five WSVs were effectively separated in less than 10 minutes, with elution order consistent with polarity sequence and plate numbers ranging from 35,000 to 58,000 plates per meter. This will further enhance the application prospects of HILIC chromatography in separating polar compounds, achieving better separation effects.

Although HPLC has the advantages of high efficiency and easy recovery, it also has the disadvantage of being difficult to simultaneously determine multiple vitamins. For example, when using HPLC to detect certain WSVs (such as thiamine), the detection limits are high, precision is low, sample storage stability is poor, and pre- or post-column fluorescence derivatization is required to improve sensitivity, inevitably involving the use of hazardous reagents [21], which is time-consuming and environmentally unfriendly. Therefore, researchers urgently need a detection method with high sensitivity and resolution, leading to the emergence of the method of coupling high-performance liquid chromatography with mass spectrometry.

3.3. Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

LC-MS/MS is an efficient and stable analytical method with unique specificity, extreme sensitivity, and the ability to analyze multiple drugs or metabolites in biological samples [42]. In LC-MS/MS, due to its wide linear dynamic range, excellent sensitivity and selectivity, and rapid data acquisition, it is widely used for absolute quantification [54]. Baiyi Lu et al. [16] utilized ultra-performance liquid chromatography-electrospray ionization-tandem triple quadrupole mass spectrometry (UPLC-ESI-MS-MS) combined with multiple reaction monitoring (MRM) mode to simultaneously determine VB_5, VB_8,

DOI: 10.54254/2753-8818/37/20240183

VB_9, and VB_{12} in infant foods. The main product ions were observed at m/z 89.9 and 202.0 for VB_5, m/z 172.0 and 227.1 for VB_8, m/z 177.0 and 295.2 for VB_9, and m/z 147.0 and 359.3 for VB_{12}, with LODs ranging from 0.005 to 0.030 μg/L and LOQs ranging from 0.016 to 0.090 μg/L. The intra-day and inter-day precision were 3.04% - 6.84% and 7.75% - 12.3%, respectively, with a recovery rate of 85.0% - 105.0%. This method exhibits high sensitivity, but it has some limitations for quantitative analysis based on full-scan acquisition [55]. Kate Porter and John K. Lodge [39] developed a simple analysis method based on hydrophilic interaction chromatography coupled with single quadrupole mass spectrometry in selected ion monitoring (SIM) mode (HILIC-MS), suitable for high-throughput analysis of WSVs in vegetable soups. The total run time of this method was 19 minutes, with a linear r2 of 0.98 - 0.99, LOD ranging from 2.4 to 9.0 ng/mL, LOQ ranging from 8 to 30 ng/mL, and intra-day and inter-day precision of 1.56% - 6.56% and 8.07% - 10.97%, respectively. Although the sensitivity of this method is lower than that of Baiyi Lu's method, the intra-day and inter-day reproducibility is better, indicating higher precision, and it is cost-effective, suitable for direct application in plant-based food matrices.

Reference [46] pointed out that LC-MS/MS method has certain matrix effects, requiring standard addition method to determine these analytes in the research matrix. Therefore, Geng Jianqiang et al. [17] used the retention time of standard substances and the ratio of qualitative and quantitative ion pairs to accurately quantify the sample peaks, overcoming the matrix effects often present in LC-MS/MS quantification. The experiment used RP-HPLC-MS method to measure the spiked recovery rate of infant formula samples, which was 85.0% - 95.5%, with an average relative standard deviation of 2.9% - 9.1%. The results were accurate and sensitive, suitable for rapid detection of large batches of samples. Melissa M. Phillips [20] adopted the liquid chromatography-isotope dilution mass spectrometry method with positive ion mode electrospray ionization technology, using internal standard method to correct errors in the analysis, thereby accurately determining the content of each vitamin in the sample. This method can be applied to various fortified and unfortified food samples, providing important data support for food safety and nutrition assessment. The laboratory of Brian A. Rappold [43] proposed that the most reliable method to address ionization matrix effects is to reduce the gradient spacing of LC and extend the running time, and to optimize additional column screening or orthogonal sample preparation techniques to improve chromatographic resolution. Thus, these methods reduce the influence of other components on the response values of analytes, thereby reducing the impact on the accuracy and repeatability of the results, and improving the precision of LC-MS/MS method.

In summary, HPLC method, due to its high sensitivity, resolution, and low cost, is suitable for frequent testing of food and environmental samples in ordinary laboratories and industries, as well as for the purification of single vitamins. In this method, RP-HPLC is more versatile, while HILIC has broader application prospects in separating polar compounds. In addition, LC-MS/MS method can provide better stability and specificity, and is more suitable for simultaneously purifying and analyzing multiple WSVs with higher precision and sensitivity, such as high-demand food analysis, drug analysis, environmental analysis, and strict control of WSV content detection.

4. Discussion

Due to the diversity of food, when using liquid chromatography, it is necessary to separate samples through extraction techniques, provide relatively pure extracts, and the separation system must be able to separate target WSVs from co-extracts, all of which require the selection of chromatographic conditions [55]. Firstly, different types of chromatographic columns have different separation advantages. Due to the high sensitivity and low cost of C_{18} columns, they are regarded as the preferred choice for detection by researchers and factories. Secondly, WSVs have good retention times at column temperatures of 25-40°C, and the mobile phase can ensure good separation of various WSVs. Finally, flow rate and elution gradient also affect the separation of vitamins, and gradient elution between 0.2-1 mL/min can meet the extraction analysis of most complex samples, while simpler samples can be tested using isocratic elution. Only when the chromatographic conditions are suitable for the analytes being measured can a solid foundation be laid for the purification and separation of WSVs.

DOI: 10.54254/2753-8818/37/20240183

In recent years, various methods have been developed for the analysis and detection of multiple WSVs in functional foods. Among them, liquid chromatography technology, with its advantages of high resolution, sensitivity, and speed, has become the most promising method for solving biochemical analysis problems, especially in the detection and application of functional foods in food analysis. High-performance liquid chromatography (HPLC) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) have been applied in food analysis at different scales due to their different functions. LC-MS/MS is a stable and efficient analytical method with advantages such as applicability to various complex matrices, extreme sensitivity, and unique specificity [40]. It is suitable for the detection of mixed other complex metabolites and meticulous sample analysis in samples, such as food quality inspection or the preparation of supplements with strict content requirements. However, this method has complex pretreatment, high cost, and is not suitable for large-scale detection. HPLC has the advantages of simple operation, good reproducibility, and high sensitivity, making it suitable for the extraction and analysis of simple samples. In comparison, the use of HPLC in large-scale testing has a higher cost-effectiveness. However, this method is limited in sensitivity and specificity to matrix effects [1], and it lacks resolution in complex foods and comprehensiveness. Therefore, Yi Yang et al. [8] developed an optimized HPLC method. They found it challenging to achieve effective baseline separation in HPLC using a single column universally. Thus, they used a mobile phase of acetonitrile-0.1% acetic acid aqueous solution (10:90) at a flow rate of 0.4 mL/min, a column temperature of 33°C, and a detection wavelength of 262 nm. Two chromatographic columns, C_{18} short column and HILIC, were coupled with different selectivities to combine the separation advantages of both, achieving effective baseline separation between target peaks and solvent peaks, and obtaining better recovery rates, limits of detection (LOD), and limits of quantification (LOQ). This optimized method is cost-effective, simple to operate, and uses one of the most common solvents in HPLC for the chromatographic mobile phase, without the need for complex sample pretreatment processes (such as solid-phase extraction). However, this method only separates one vitamin, VB_3, and there is still a need for a method that can purify and detect most WSVs. Mustafa Abdullah Yilmaz et al. [40] proposed an updated LC-MS/MS method. They compensated for sensitivity loss and improved precision by using isotope-labeled vitamin standards. They performed gradient elution at a flow rate of 0.4 mL/min with a mobile phase of water-methanol containing 10 mM ammonium acetate and 0.1% acetic acid on a C_{18} column, achieving optimal separation of nine WSVs except for VB_7, purification of almost all WSVs, and applicability to the detection of various natural foods. Therefore, the optimized HPLC method can be used for large-scale analysis of simple samples, and the updated LC-MS/MS method can be used for high-demand purification of complex samples. Additionally, the HILIC-MS method used by Kate Porter and John K. Lodge [39] also has great applicability in the precision and cost of extraction and analysis of WSVs in functional foods.

In conclusion, selecting appropriate chromatographic conditions and corresponding liquid chromatography techniques according to different foods can achieve better purification effects and detection results of WSVs.

5. Discussion

Given the highly complex nature of WSVs in functional foods, there are currently no universally applicable chromatographic conditions for such substances. Among them, using a C18 chromatographic column at 25-40°C and a mobile phase consisting of buffer solution-methanol/acetonitrile system with gradient elution at a flow rate of 0.2-1 mL/min, multiple WSVs in complex functional foods can be simultaneously detected using liquid chromatography. The choice of mobile phase needs to be comprehensively considered based on the characteristics of the samples themselves, such as the pH of the mobile phase, whether to add ion pairing reagents, and the concentration of additives. The flow rate and elution gradient need to be determined based on the complexity of the sample matrix and the types of WSVs present. Specific liquid chromatographic methods should be selected according to actual requirements. For example, in ordinary laboratories and factories, RP-HPLC is more suitable for large-scale analysis and detection of simple samples. If its chromatographic behavior is unsatisfactory,

DOI: 10.54254/2753-8818/37/20240183

coupling or using HILIC or other detectors for analysis can be attempted. For high-demand food and drug production analysis or precision experiments, LC-MS/MS can be used for sample purification and detection. In the future, the use of a C_{18} column coupled with an HILIC column can provide a low-cost and simple method with high resolution results for the detection of some WSVs in food. For the simultaneous purification of multiple WSVs in complex matrices, HILIC-MS and UPLC-MS/MS will provide better separation efficiency, offering new research methods for food quality inspection and the production of targeted supplements.

References

[1] Sim H J, Kim B and Lee J (2016). A Systematic Approach for the Determination of B-Group Vitamins in Multivitamin Dietary Supplements by High Performance Liquid Chromatography with Diode-Array Detection and Mass Spectrometry. Journal of Aoac International. 99(5), 1223-32.

[2] Karatapanis A E, Flamegos Y C and Stalikas C D (2009). HILIC separation and quantitation of water-soluble vitamins using diol column. Journal of Separation Science. 32(7), 909-17.

[3] Mateeva A, Kondeva-Burdina M, Peikova L, Guncheva S, Zlatkov A and Georgieva M (2023). Simultaneous analysis of water-soluble and fat-soluble vitamins through RP-HPLC/DAD in food supplements and brewer's yeast. Heliyon. 9(1), e12706.

[4] Wills R, Shaw C and Day W (1977). Analysis of water soluble vitamins by high pressure liquid chromatography. Journal of Chromatographic Science. 15(7), 262-6.

[5] Wardenaar F C, Ceelen I J, Van Dijk J-W, Hangelbroek R W, Van Roy L, Van der Pouw B, De Vries J H, Mensink M and Witkamp R F (2017). Nutritional supplement use by Dutch elite and sub-elite athletes: does receiving dietary counseling make a difference? International Journal of Sport Nutrition and Exercise Metabolism. 27(1), 32-42.

[6] Nguyen H D, Oh H and Kim M-S (2022). Mixtures modeling identifies vitamin B1 and B3 intakes associated with depression. Journal of Affective Disorders. 301, 68-80.

[7] Luo Y and Guo Y (2023). Nanomaterials for fluorescent detection of vitamin B2: A review. Analytical Biochemistry. 683, 115351.

[8] Yang Y, Shi K, Xu S, Tian Y, Huang H, Cai X, Liu Z, Xu Z, Zhang F and He P (2023). Dual-Chromatographic Column Tandem Technique for Optimizing Separation and Determination of Vitamin B3. Food Analytical Methods. 16(9), 1468-76.

[9] Metsu D, Caspar-Bauguil S and Galinier A (2023). Carences et excès en micronutriments. Nutrition Clinique et Métabolisme. 37(3), 130-48.

[10] Munteanu C, Berindean I, Mihai M, Pop B, Popa M, Muntean L, Petrescu O and Ona A (2023). E, K, B5, B6, and B9 vitamins and their specific immunological effects evaluated by flow cytometry. Frontiers in Medicine. 9, 1089476.

[11] Solvik B S and Strand T A (2024). Biotin: a scoping review for Nordic Nutrition Recommendations 2023. Food & nutrition research. 68.

[12] Fan B, You J, Suo Y and Qian C (2018). A novel and sensitive method for determining vitamin B3 and B7 by pre-column derivatization and high-performance liquid chromatography method with fluorescence detection. Plos one. 13(6), e0198102.

[13] Vincenti A, Bertuzzo L, Limitone A, D'Antona G and Cena H (2021). Perspective: Practical approach to preventing subclinical b12 deficiency in elderly population. Nutrients. 13(6), 1913.

[14] Chen C, wei X, Cai W, Li X and Zhang X (2017). Determination of pantothenate, nicotinamide, vitamin B1, vitamin B2, and vitamin B6 in healthy food by high performance liquid chromatography. Journal of Food Safety and Quality. 8(5), 1866-71.

[15] Dodevska M, Milenkovic M, Jovanovic V and Ivanovic N (2023). Analysis of vitamin content in products intended for infants and children on the Serbian market. Journal of Food Composition and Analysis. 123, 105633.

[16] Lu B, Ren Y, Huang B, Liao W, Cai Z and Tie X (2008). Simultaneous determination of four water-soluble vitamins in fortified infant foods by ultra-performance liquid chromatography

DOI: 10.54254/2753-8818/37/20240183

coupled with triple quadrupole mass spectrometry. Journal of chromatographic science. 46(3), 225-32.

[17] JianQiang G, Li Z, HongYan P, MingYuan S and Hao W (2017). Study on simultaneous detection of 12 Water-soluble Vitamins in infant milk powder by HPLC-MS. China Dairy Industry. 45(08), 43-6.

[18] Zhang S Q, Li J, Li L, Yuan X T, Xu L and Shi Z G (2020). Fast separation of water-soluble vitamins by hydrophilic interaction liquid chromatography based on submicrometer flow-through silica microspheres. Food Chemistry. 307, 125531.

[19] Langer S and Lodge J K (2014). Determination of selected water-soluble vitamins using hydrophilic chromatography: A comparison of photodiode array, fluorescence, and coulometric detection, and validation in a breakfast cereal matrix. Journal of Chromatography B. 960, 73-81.

[20] Phillips M M (2015). Liquid chromatography with isotope-dilution mass spectrometry for determination of water-soluble vitamins in foods. Analytical and Bioanalytical Chemistry. 407, 2965-74.

[21] Ivarsen E, Andersen C P, Jensen S M, Pedersen C T and Svaneborg A K (2021). Quantification of retinyl palmitate, thiamine, niacin, pyridoxine, folic acid, cyanocobalamin, zinc, and iron by chromatographic methods in fortified kernels and fortified rice. Acta Chromatographica. 33(2), 145-52.

[22] Vella M N, Stratton L M, Sheeshka J and Duncan A M (2014). Functional food awareness and perceptions in relation to information sources in older adults. Nutrition journal. 13, 1-12.

[23] Yue-e W, Dong-xia L, Zhi L and Fang-rong H (2020). Identification of Vitamin B using a quad-peak terahertz metamaterials sensor. Guangpuxue yu Guangpu Fenxi/Spectroscopy and Spectral Analysis, 1785-90.

[24] Kumar R, Singh U, Tiwari A, Tiwari P, Sahu J K and Sharma S (2023). Vitamin B12: Strategies for enhanced production, fortified functional food products and health benefits. Process Biochemistry. 127, 44-55.

[25] Gheorghe D C, Stefan-van Staden R I and van Staden J F (2024). Mini-Review: Electrochemical Sensors Used for the Determination of Water- and Fat-Soluble Vitamins: B, D, K. Critical Reviews in Analytical Chemistry. 54(1), 1-10.

[26] Bolshakov D S and Amelin V G (2023). Capillary Electrophoresis in Assessing the Quality and Safety of Foods. Journal of Analytical Chemistry. 78(7), 815-55.

[27] Neamtu A Set al. (2020). Simultaneous Quantitation of Nicotinamide Riboside and Nicotinamide in Dietary Supplements via HPTLC-UV with Confirmation by Online HPTLC-ESI-MS. Acta Chromatographica. 32(2), 128-33.

[28] Kim H J (2016). Development of an ion-pairing reagent and HPLC-UV method for the detection and quantification of six water-soluble vitamins in animal feed. International Journal of Analytical Chemistry. 2016, 8357358.

[29] Kadakal Ç, Ekinci R and Yapar A (2007). The effect of cooking and drying on the water-soluble vitamins content of bulgur. Food science and technology international. 13(5), 349-54.

[30] Evers M S, Alexandre H, Morge C, Sparrow C, Gobert A and Roullier-Gall C (2023). Exploring the unexplored: A characterization of vitamins and vitamers in white grape musts by high-performance liquid chromatography. Food Chemistry. 398, 133860.

[31] Aslam J, Khan S H and Khan S A (2013). Quantification of water soluble vitamins in six date palm (Phoenix dactylifera L.) cultivar's fruits growing in Dubai, United Arab Emirates, through high performance liquid chromatography. Journal of Saudi Chemical Society. 17(1), 9-16.

[32] Niu C, Ye S, Hu Y, Wang F and Ling Y (2022). Rapid determination of seven water-soluble vitamins in starch-fortified foods. Food Science. 43(24), 232-8.

[33] Ciulu M, Solinas S, Floris I, Panzanelli A, Pilo M I, Piu P C, Spano N and Sanna G (2011). RP-HPLC determination of water-soluble vitamins in honey. Talanta. 83(3), 924-9.

DOI: 10.54254/2753-8818/37/20240183

[34] Ibrahim L and Usman A (2022). A simple and rapid RP-HPLC method for the assessment of cobalamin (vitamin B12) intilapia and snoek fishes. Turkish Journal of Chemistry. 46(2), 320-9.
[35] Patle T K, Shrivas K, Patle A, Patel S, Harmukh N and Kumar A (2022). Simultaneous determination of B1, B3, B6 and C vitamins in green leafy vegetables using reverse phase-high performance liquid chromatography. Microchemical Journal. 176, 107249.
[36] Chutkowski M, Ziobrowski P, Przywara M, Kamińska J and Zapała W (2022). Studies on the Effects of Process Conditions on Separation of B1, B2 and B3 Vitamin Mixture Using HILIC and RPLC Chromatography. AgriEngineering. 4(3), 566-91.
[37] Islam M A, Park E, Jeong B, Gwak Y-J, Kim J, Hong W-H, Park S-J, Jung J, Yoon N-Y and Kim Y-K (2022). Validation of vitamin B5 (pantothenic acid) and B6 (pyridoxine, pyridoxal, and pyridoxamine) analyses in seafood. Journal of Food Composition and Analysis. 109, 104518.
[38] Yan H, Cui F, Bie W, Qi H, Feng X and Zhang Z (2020). Simultaneous determination of 10 kinds of water soluble vitamins in infant formula by ultra performance liquid chromatography–isotope dilution mass spectrometry. Journal of Food Safety & Quality. 11(17), 5871-8.
[39] Porter K and Lodge J K (2021). Determination of selected water-soluble vitamins (thiamine, riboflavin, nicotinamide and pyridoxine) from a food matrix using hydrophilic interaction liquid chromatography coupled with mass spectroscopy. Journal of Chromatography B. 1171, 122541.
[40] Yilmaz M A, Cakir O and Yener I (2023). A fast, accurate and comprehensive LC-MS/MS method validation for the sensitive quantification of water-soluble vitamins in walnut, almond, hazelnut and pistachio fruits. International Journal of Mass Spectrometry. 488, 117040.
[41] Paglia G and Astarita G (2022). A High-Throughput HILIC-MS-Based Metabolomic Assay for the Analysis of Polar Metabolites. Plant Metabolic Engineering: Methods and Protocols, 137-59.
[42] Geng C M, Guo X, Liu J J, Gao M M, Yuan G Y, Bu F L, Chen X W, Wang B J and Guo R C (2017). LC-MS/MS for the Determination of Four Water-Soluble Vitamins: Method Development, Validation and Comparison to EC Method. Chromatographia. 80(2), 259-64.
[43] Rappold B A (2022). Review of the use of liquid chromatography-tandem mass spectrometry in clinical laboratories: Part I-Development. Annals of laboratory medicine. 42(2), 121-40.
[44] Rappold B A (2022). Review of the use of liquid chromatography-tandem mass spectrometry in clinical laboratories: Part II-Operations. Ann Lab Med. 42(5), 531-57.
[45] Chatterjee N S, Kumar K A, Ajeeshkumar K K, Kumari K R R, Vishnu K V, Anandan R, Mathew S and Ravishankar C N (2017). Screening Natural Content of Water-Soluble B Vitamins in Fish: Enzymatic Extraction, HILIC Separation, and Tandem Mass Spectrometric Determination. Journal of Aoac International. 100(3), 579-85.
[46] Fenoll J, Martinez A, Hellin P and Flores P (2011). Simultaneous determination of ascorbic and dehydroascorbic acids in vegetables and fruits by liquid chromatography with tandem-mass spectrometry. Food Chemistry. 127(1), 340-4.
[47] Cheng X Q, Ma D, Fei G Q, Ma Z Y, Xiao F, Yu Q J, Pan X L, Zhou F, Zhao L and Zhong C J (2018). A single-step method for simultaneous quantification of thiamine and its phosphate esters in whole blood sample by ultra-performance liquid chromatography-mass spectrometry. Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences. 1095, 103-11.
[48] Wu H, Zheng A, Su C, Yu N and Li N (2017). Simultaneous separation of seven water-soluble vitamins with microemulsion liquid chromatography. Chinese Journal of Chromatography. 35(4), 439.
[49] Plozza T, Trenerry V C and Caridi D (2012). The simultaneous determination of vitamins A, E and β-carotene in bovine milk by high performance liquid chromatography–ion trap mass spectrometry (HPLC–MSn). Food Chemistry. 134(1), 559-63.

DOI: 10.54254/2753-8818/37/20240183

[50] Akash M S H, Rehman K, Akash M S H and Rehman K (2020). High performance liquid chromatography. Essentials of pharmaceutical analysis, 175-84.
[51] Chamseddin C and Jira T (2013). Evaluation of the chromatographic performance of conventional, polar-endcapped and calixarene-bonded stationary phases for the separation of water-soluble vitamins. Chromatographia. 76(9-10), 449-57.
[52] Jandera P (2012). Programmed elution in comprehensive two-dimensional liquid chromatography. Journal of Chromatography A. 1255, 112-29.
[53] Geng H, Jing J, Zhang F, Zhang F and Yang B (2020). A polar stationary phase obtained by surface-initiated polymerization of hyperbranched polyglycerol onto silica. Talanta. 209, 120525.
[54] Lyon D, Weckwerth W and Wienkoop S (2014). Mass Western for absolute quantification of target proteins and considerations about the instrument of choice. Plant Proteomics: Methods and Protocols, 199-208.
[55] Nikolin B, Imamović B, Medanhodžić-Vuk S and Sober M (2004). High perfomance liquid chromatography in pharmaceutical analyses. Bosnian journal of basic medical sciences. 4(2),