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A CRITICAL REVIEW OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY IN THE ANALYSIS OF WATER-SOLUBLE VITAMINS

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This review presents an in-depth analysis of the advancements in High-Performance Liquid Chromatography (HPLC) for detecting and measuring water-soluble vitamins, such as thiamine (B1), riboflavin (B2), pyridoxal (B6), and cobalamin (B12), from 2000 to 2024. These vitamins play essential roles in metabolic functions, energy synthesis, and antioxidant defense mechanisms, making their accurate quantification necessary for food and pharmaceutical industries. Their instability under environmental conditions, including heat, light, and oxygen exposure–further emphasizes the demand for precise detection methods. HPLC has become the preferred analytical technique due to its high sensitivity, selectivity, and ability to analyze complex food compositions. This review highlights significant improvements in chromatographic separation techniques and detection methods that have enhanced accuracy, reproducibility, and reliability in vitamin analysis. Innovations in detector technologies have minimized matrix interferences, leading to more dependable results. Ongoing improvements in HPLC are essential to meet the increasing demand for precise vitamin quantification. This study underscores HPLC's evolution as a critical tool in ensuring food quality and safety.

Keywords: Chromatographic separation, Food and pharmaceutical analysis, High-Performance Liquid Chromatography (HPLC), Vitamin quantification, Water-soluble vitamins.

INTRODUCTION

Water-soluble vitamins, including thiamine (B1), riboflavin (B2), pyridoxal (B6), and cobalamin (B12) (Fig. 1), play essential roles in human health. They act as cofactors in metabolic reactions and contribute to antioxidant defense. Their significance has led to increased demand in the food and pharmaceutical industries, resulting in the widespread availability of fortified foods and dietary supplements. Given their biological importance, accurate measurement of these vitamins in food and pharmaceutical products is essential.

Water-soluble vitamins degrade under environmental factors such as heat, light, oxygen, and pH variations, complicating their detection. Therefore, analytical methods must prevent degradation and accurately measure all vitamin forms present in food and pharmaceutical samples. Studies from the U.S.

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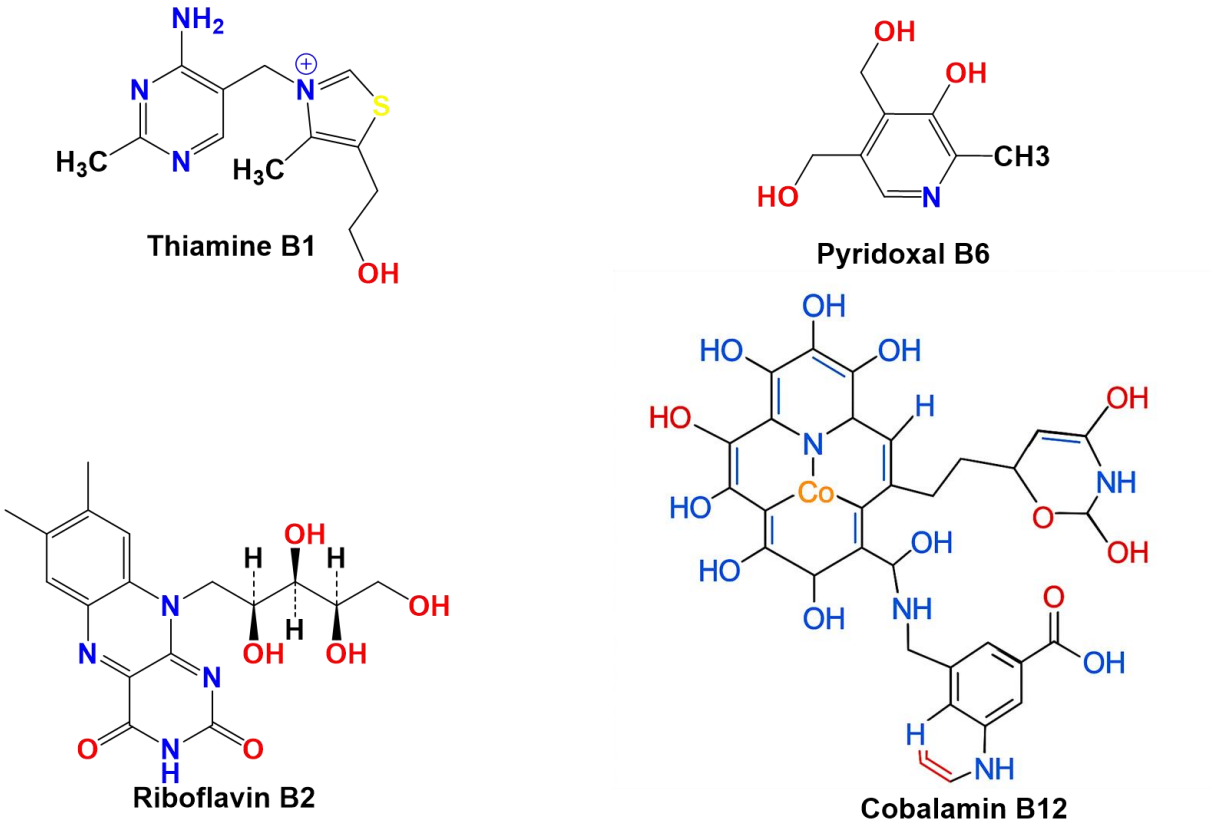

Fig. 1: Chemical structures of vitamins studied.

National Institute of Standards and Technology (NIST) highlight that certain vitamins degrade when exposed to air, heat, or light. Ensuring stability throughout the analytical process is crucial for precise results[1].

Analytical methods for water-soluble vitamins must be robust, reliable, and efficient, allowing for quick, routine, and cost-conscious analyses. Ensuring that analytes remain stable during the entire process is vital, and both extraction and chromatographic procedures should be precise and reproducible. Accuracy is paramount to limit uncertainty, and the detector must be sufficiently selective to avoid interferences. Controlling matrix effects is also crucial to prevent skewed results. Despite these requirements, analyzing water-soluble vitamins in foods is particularly demanding because of their inherent instability. Aging, storage, and processing can all influence vitamin degradation, as temperature, oxygen, light, moisture, and pH each play a role in potential losses. Consequently, methods must fully extract and measure all naturally occurring vitamin forms. Research by the U.S. National Institute of Standards and Technology (NIST) underscores the tendency of certain antioxidants (including some vitamins) to degrade when exposed to air, heat, or light. As a result, maintaining analyte stability throughout analysis is essential for achieving accurate results[2].

Although several analytical techniques exist for detecting water-soluble vitamins, high-performance liquid chromatography (HPLC) has gained prominence due to its superior sensitivity, specificity, and adaptability. For reliable vitamin analysis, methods must ensure precision, reproducibility, and minimal interference from other compounds in complex food matrices. The efficiency of HPLC allows for accurate quantification, even in challenging samples, making it a preferred choice for vitamin analysis.

Recent advances in HPLC, including the use of ultra-high-performance liquid chromatography (UHPLC), diode array detection (DAD), tandem mass spectrometry (LC-MS/MS), and solid-phase extraction

(SPE), have dramatically improved the resolution, sensitivity, and selectivity in water-soluble vitamin analysis. These methods allow for simultaneous quantification of structurally similar vitamers, even in highly complex food and pharmaceutical matrices. For example, methods combining SPE with fluorometric or electrochemical detection have minimized matrix effects and achieved low detection limits suitable for trace-level analysis.

This is particularly crucial for vitamins like B6, which exists in structurally similar forms (pyridoxal, pyridoxamine, and pyridoxine), each with varying stability and bioactivity. Analytical methods employing pre-column derivatization and optimized mobile phase systems can efficiently resolve these vitamers in complex matrices such as cereal-based infant formulas, dairy, or nutraceutical products[3]. In samples like milk, fortified cereals, and energy drinks, vitamins are often present at trace levels and embedded within challenging matrices. HPLC's ability to quantify analytes at nanomolar concentrations even in the presence of fats, proteins, and preservatives makes it indispensable for nutritional labeling, quality assurance, and clinical diagnostics. For example, reversed-phase HPLC coupled with fluorescence or MS has achieved detection limits as low as 0.01 µg/g for B1 and B2 vitamens in processed foods and beverages[4].

1. Thiamine (B1)

Thiamine, commonly known as vitamin B1 (Fig. 1), is a water-soluble vitamin essential for carbohydrate metabolism and nervous system function. It consists of a pyrimidine ring linked to a thiazole ring through a methylene bridge. Despite its importance, the human body cannot synthesize thiamine, requiring intake from dietary sources. The active form, thiamine diphosphate (ThDP), serves as a coenzyme in several metabolic pathways, particularly those involved in energy production. A deficiency in vitamin B1 disrupts key metabolic functions, affecting glucose metabolism, mitochondrial activity, and neurotransmitter synthesis. Insufficient thiamine levels lead to conditions such as beriberi, Wernicke–Korsakoff syndrome, and neurodegenerative diseases like Alzheimer's and Parkinson's. Deficiency can result from poor dietary intake or medical conditions that impair absorption[5&6].

Accurate quantification of thiamine in food and supplements is critical for ensuring adequate intake. Various analytical methods exist, but HPLC remains the most widely used technique due to its precision and ability to detect thiamine at low concentrations. Research suggests that cooking and storage conditions significantly impact thiamine levels, with raw foods generally containing higher amounts compared to processed or stored products.

B1 plays a fundamental role in human physiology and has been extensively investigated by medical and nutritional scientists. As a cofactor for multiple enzymes, it is integral to cellular metabolism, influencing both direct and indirect biochemical pathways. Insufficient dietary intake of thiamine disrupts key metabolic processes, including glucose utilization, energy production, and mitochondrial function. Deficiency can result in lactic acidosis due to pyruvate dehydrogenase (PDH) impairment, compromise DNA synthesis by diminishing transketolase activity and ribose-5-phosphate availability within the pentose phosphate pathway, and adversely affect neurotransmitter biosynthesis[7].

Thiamine deficiency presents clinically through several conditions, most notably beriberi, a cardiomyopathy characterized by edema and lactic acidosis, and Wernicke–Korsakoff syndrome, also referred to as Wernicke's encephalopathy. Additionally, insufficient thiamine levels have been linked to the pathogenesis of multiple neurodegenerative disorders, including Alzheimer's, Parkinson's, and Huntington's disease. As an essential nutrient, thiamine plays a critical role in maintaining neurological and metabolic health, and its deficiency has also been associated with certain developmental disorders, such as autism[8].

B1 deficiency can result from insufficient dietary intake or medical conditions that impair absorption. Maintaining adequate levels typically depends on a well-balanced diet, with supplementation when needed. Accurate and efficient analytical methods are essential for assessing vitamin content in foods and supplements to ensure proper intake. Among

available techniques, chromatographic methods, especially high-performance liquid chromatography (HPLC), are the most employed for quantifying vitamin B1[6].

Many laboratories focus on analyzing raw ingredients, while fewer studies delve into the nutrient content of foods in their cooked or consumed form. Typically, raw foods display higher nutrient values, as cooking and storage can lead to vitamin loss (Table 1).

Table 1: Thiamine Content in Various Foods[8].

Food source	Thiamine content (mg per 100 g)
Whole Grains (Brown Rice, Oats)	0.45 – 1.2
Fortified Cereals	1.0 – 2.5
Legumes (Lentils, Peas)	0.3 – 0.6
Pork (Lean Cuts)	0.8 – 1.1
Nuts (Sunflower Seeds, Macadamia)	0.5 – 1.2
Fish (Tuna, Salmon)	0.2 – 0.4
Dairy (Milk, Yogurt)	0.02 – 0.05
Vegetables (Spinach, Asparagus)	0.05 – 0.1
Fruits (Oranges, Bananas)	0.03 – 0.05

1.1. Biosynthesis

Thiamine biosynthesis occurs through two separate pathways that produce its key structural components, the pyrimidine and thiazole moieties. These components are synthesized independently and later combined by specific enzymes. In bacteria such as Escherichia coli and Bacillus subtilis, thiamine biosynthesis follows well-characterized pathways with slight variations. Recent studies have standardized gene nomenclature across bacterial species, improving our understanding of thiamine synthesis[9]. In all organisms, thiamin monophosphate (ThMP) is synthesized through separate pathways for its thiazole and pyrimidine components, which are later joined by a coupling enzyme. ThMP is then phosphorylated by a specific kinase to form thiamin diphosphate (ThDP), the biologically active cofactor. The biosynthesis of thiamin has been extensively characterized in *Escherichia coli* and *Bacillus subtilis*. While these pathways share fundamental similarities, they also exhibit distinct differences. Figure 2 presents the enzymes responsible for thiamin biosynthesis in prokaryotes along with their corresponding gene names. Previously, gene nomenclatures differed between *E. coli* and *B. subtilis*, but a standardized system has since been established for both species.

1.2. Pharmacokinetics[10]

- **Absorption:** Thiamine from food must be converted into its free form before being absorbed in the small intestine. At typical dietary levels, active transport mechanisms facilitate absorption, whereas at higher doses, passive diffusion plays a greater role.
- **Distribution:** Once absorbed, thiamine is phosphorylated and transported to various organs, including the liver, heart, brain, and kidneys.
- **Metabolism:** The primary metabolized form is thiamine diphosphate, which functions as a coenzyme in carbohydrate metabolism.
- **Elimination:** Excess thiamine is excreted through urine due to its water-soluble nature.

1.3. Pharmacodynamics

Thiamine contributes to antioxidant defense, cognitive function, cardiovascular health, and detoxification processes. It plays a vital role in intracellular glucose metabolism, reducing oxidative stress and preventing cellular damage. Studies have shown that thiamine protects against lipid peroxidation induced by toxins such as lead, further emphasizing its role in cellular protection[6&10].

HPLC has become a key method for analyzing thiamine levels in biological samples and food products. The technique's high sensitivity and selectivity enable accurate detection even in complex matrices. Sample preparation methods, such as solid-phase extraction (SPE), enhance the efficiency of thiamine analysis by removing interferences. However, method reproducibility remains a challenge, requiring validation procedures for consistency.

Association of Official Analytical Collaboration (AOAC) approved methods for thiamine detection involve oxidation reactions with hexacyanoferrate (III), followed by fluorometric quantification. While effective, these methods require optimization for different food matrices to ensure reliable results. The ongoing development of HPLC techniques continues to improve accuracy, efficiency, and sensitivity in thiamine analysis.

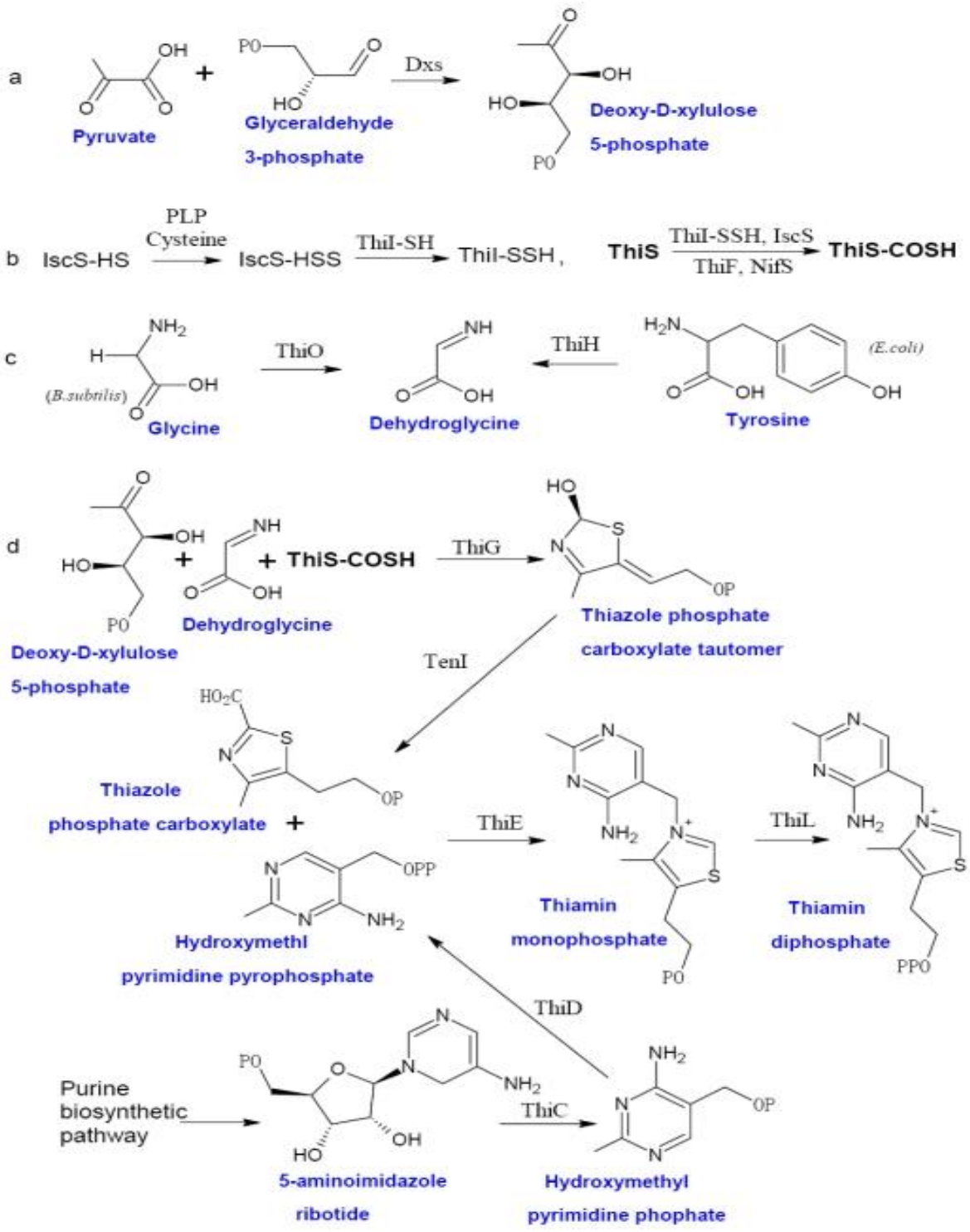

Fig. 2: Complete *de-novo* thiamine biosynthetic pathway in bacteria[9].

High-performance liquid chromatography (HPLC) remains a crucial tool for analyzing biologically labile compounds, including water-soluble vitamins. Advanced instrumentation and optimized column packings enhance the precision and sensitivity of these analyses. However, developing methodologies for complex sample matrices, such as food and dietary products, increasingly emphasizes effective sample pretreatment and purification strategies. Solid-phase extraction (SPE) enables highly selective separation based on specific interactions between analytes and the stationary phase, improving efficiency by

minimizing analysis time, cleanup steps, and solvent consumption. Automated systems further streamline these processes, though concerns about SPE reproducibility persist. Implementing systematic method validation, including matrix compatibility assessments, stationary phase screening, and test runs for each new cartridge lot, can address these limitations.

The AOAC Official Method 942.23[11] for thiamine (Vitamin B_1) determination in food products involves the oxidation of thiamine to thiochrome using alkaline potassium hexacyanoferrate (III), followed by liquid–liquid extraction of the fluorescent thiochrome derivative into an organic solvent such as isobutanol for subsequent fluorometric quantification[11]. Despite its widespread adoption, this method is susceptible to matrix-dependent variability due to incomplete or inefficient thiochrome formation, potentially compromising analytical accuracy across complex food substrates. To assess and improve analytical recovery, the C technique is employed to monitor potential thiamine degradation products and incomplete conversions. Furthermore, advanced methodologies utilizing stable isotope-labeled thiamine have been developed to trace its metabolic derivatives, including thiamine phosphate esters, and to study absorption, distribution, and biochemical transformations in biological systems[12]. In parallel, endogenous labeling approaches have proven effective for evaluating extraction efficiency and bioavailability of native thiamine in fortified and natural matrices[13].

Table 2 presents recent high-performance liquid chromatography (HPLC) methods used for analyzing thiamine (Vitamin B1) in food samples, highlighting advancements in chromatographic techniques designed for precision and efficiency. It details various mobile phase compositions, stationary phases, and detection systems that enhance sensitivity and selectivity. Key developments include the application of reverse-phase columns with gradient elution, frequently combined with fluorescence or UV-Vis detection to improve quantification accuracy. These methods demonstrate versatility across diverse food matrices (Fig. 3)[14], including cereals, beverages, and fortified products, emphasizing robust sample preparation and effective management of matrix interferences. The continuous refinement of these techniques underscores the significance of method optimization in ensuring accurate and reliable thiamine measurement in complex food systems.

Table 2: Recent HPLC methods for thiamine (vitamin B1) analysis in food samples.

Sample	Column	Mobile phase	Detection	Ref.
Salmon, oysters, mussels, pig liver	C_{18} (5 µm, 25 cm × 4.6 mm)	Methanol: Phosphate buffer (10:90) with 0.018 M trimethylamine, pH 3.55	Electrochemical, DAD/UV, Coulometric	4
Fruit juices, apple juice, fortified fruit juice	C_{18} (5 µm, 25 cm × 4.6 mm)	Methanol: Phosphate buffer (10:90) with 0.018 M trimethylamine, pH 3.55	Electrochemical, DAD/UV, Coulometric	15
Maize starch pudding (Mahalabia), homemade bread (Battawi)	C_{18} stainless steel (25 cm × 4 mm, i.d.)	5% Methanol: 95% Ion-pair reagent	Fluorescence (λ_{ex} 360 nm, λ_{em} 436 nm)	16
Honey	C_{18} (10 µm, 150 × 3.9 mm)	Sulfuric acid (aqueous) and 2% (v/v) methanol	Programmable UV, Fluorescence	17
Wheat flours	Hypersil C18 (100 × 4.6 mm, 5 µm)	0.02 M phosphate buffer in 88% methanol and 12% acetonitrile	Fluorescence	18
Energy drinks	Nova-Pak C_{18} (150 mm × 3.9 mm, 5 µm)	Gradient: Methanol (Solvent A), 0.05 M NaH_2PO_4 with 0.005 M hexanesulfonic acid, pH 3.0 (Solvent B)	Fluorescence	19

Table 2: Continued.

Sample	Column	Mobile phase	Detection	Ref.
Edible marine seaweeds (Porphyra sp., powdered U. pinnatifida)	Waters C18 Sep-Pak	0.005 M Ammonium acetate: Methanol (72:28, v/v)	Fluorescence	20
Bovine milk (Fig. 3)	Acquity UPLC™ with HSS T3 (100 mm × 2.1 mm, 1.7 µm)	Sodium acetate trihydrate (99.5%)	UPLC	14
Fresh pig liver	C18	Phosphate buffer (pH 3.0): CAN (4:16, v/v) with 5 mM sodium heptanesulfonate	UV (254 nm)	21
Traditional Turkish cereal food	C18 (150 mm × 4.6 mm)	0.1 M KH_2PO_4 (pH 7.0): Methanol (90:10, v/v)	UV–Vis DAD	3
Dairy products, fruit juices, cereal products	C18 Nova-Pack (250 × 4.6 mm, 5 µm)	70% Buffer (sodium salt of hexane sulfonic acid) and 30% methanol	UV	22

Fig. 3: Graphical abstract of HPLC determination of thiamine in bovine milk[14].

2. Riboflavin (B2)

Riboflavin (7,8-dimethyl-10-ribityl-iso-alloxazine) (Fig. 1) is a water-soluble vitamin found in various dietary sources. Initially identified in 1879 as "lactochrome" from milk whey, it was not fully purified at the time. In its pure form, riboflavin crystallizes into orange-yellow solids with limited solubility in water. Its biologically active derivatives, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), serve as essential cofactors in redox reactions necessary for aerobic metabolism. Despite its essential role, riboflavin deficiency remains prevalent in specific populations, including some within well-nourished societies. Research on the physiological consequences of deficiency is still relatively limited. However, increasing interest in its potential protective effects against cancer and cardiovascular disease has underscored the need to reassess its metabolic functions and public health implications. Despite its essential role, riboflavin deficiency remains prevalent in specific populations, including some within well-nourished societies. Research on the physiological consequences of deficiency is still relatively limited. However, increasing interest in its potential protective effects against cancer and cardiovascular disease has underscored the need to reassess its metabolic functions and public health implications (Table 3)[23].

Riboflavin deficiency is particularly common in populations with limited dairy and meat consumption. In Guatemala, studies have shown a strong correlation between riboflavin

status in older adults and milk consumption. Data from the UK National Diet and Nutrition Survey of individuals aged 4-18 years reveal a high prevalence of biochemical riboflavin deficiency, particularly among adolescent girls. A progressive decline in whole milk consumption has been observed with age in both sexes. Moreover, the latest UK National Food Consumption Survey reports a continued decrease in household purchases of liquid whole milk, with a 47% reduction since 1990[24].

The consumption of semi-skimmed and other skimmed milk varieties has risen, partly offsetting shifts in dietary patterns, whereas fully skimmed milk remains less widely consumed. Naturally low in riboflavin, grain-based foods have become significant dietary sources due to fortification efforts, particularly in certain breads and cereals. In the United Kingdom, fortified cereals contribute over 20% of household riboflavin intake. Regular consumption of breakfast cereal with milk is therefore an effective strategy for maintaining adequate riboflavin levels. Studies conducted in various countries consistently indicate that individuals across all age groups who consume breakfast cereal tend to have higher riboflavin intake and status than those who do not[25].

2.1. Biosynthesis

The biosynthesis of riboflavin involves multiple enzymatic reactions, each responsible for specific molecular transformations. The pathway begins with the enzyme GTP cyclohydrolase II, which initiates a sequence of biochemical modifications leading to riboflavin formation. The process includes steps such as deamination, reduction, and phosphorylation, resulting in the production of riboflavin, FMN, and FAD.

Different species exhibit variations in riboflavin biosynthesis, with bacteria, plants, and fungi following slightly different pathways. For example, eubacteria and plants utilize one set of biosynthetic steps, while fungi and archaea employ alternative enzymatic routes. These differences highlight evolutionary divergence in riboflavin metabolism.

Table 3: Riboflavin content of some foods[26].

Food and Description	Riboflavin (mg/100 g)	Energy (kcal/100 g)
Yeast, baker's dry (active)	5.41	282
Liver, lamb, broiled	5.11	261
Yeast, torula	5.06	277
Kidneys, beef, braised	4.58	252
Liver, hog, fried in margarine	4.36	241
Yeast, brewer's, debittered	4.28	283
Liver, beef or calf, fried	4.18	242
Brewer's yeast, tablet form	4.04	—
Cheese	3.53	375
Turkey, giblets, cooked (gizzard fat)	2.72	233
Kidneys, lamb, raw	2.42	105
Kidneys, calf, raw	2.40	113
Eggs, chicken, dried, white powder	2.32	372
Whey, sweet, dry	2.21	354
Eggs, chicken, dried, white flakes	2.16	351
Liver, turkey, simmered	2.13	230
Whey, acid dry	1.89	195
Heart, hog, braised	1.80	209
Milk, cow's dry, skim, solids	1.78	353
Liver, chicken, simmered	1.75	165
Liver, beef or calf, fried	4.18	242

Riboflavin kinase catalyzes the phosphorylation of riboflavin, producing flavin mononucleotide (FMN) in Step X. Subsequently, FMN undergoes conversion to flavin adenine dinucleotide (FAD) through the action of FAD synthetase in Step XI. This biosynthetic pathway incorporates several intermediates, including GTP (1), 2,5-diamino-6-ribosylamino-4(3H)-pyrimidinone 5′-phosphate (2), and ribulose 5-phosphate (7), alongside essential metabolites such as riboflavin (10), FMN (11), and FAD (12). Notably, the pathway demonstrates significant variation across biological domains. Eubacteria and plants follow one route (green), while fungi and archaea utilize a distinct pathway (blue). Additionally, the metabolic processing of the four-carbon precursor originating from ribulose 5-phosphate (red) underscores evolutionary divergence in flavin biosynthesis (Fig. 4)[27&28].

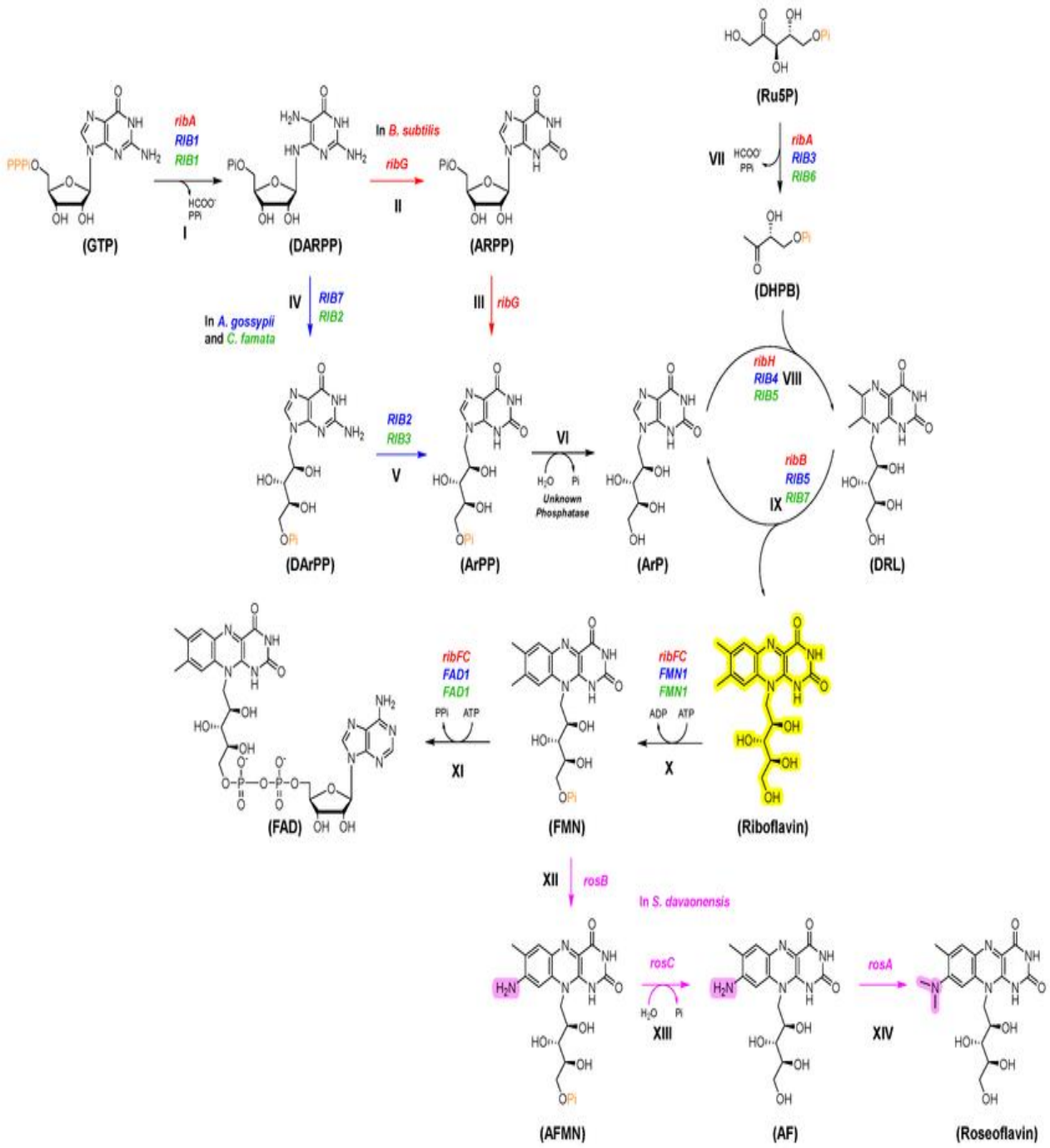

Fig. 4: Biosynthesis of riboflavin[27&28].

2.2. Pharmacokinetics

- **Absorption:** Dietary riboflavin exists primarily in bound forms, predominantly as flavin adenine dinucleotide (FAD) and, to a lesser extent, as flavin mononucleotide (FMN), with only a small fraction present in its free form. The intestinal microbiota contribute to endogenous riboflavin synthesis. Prior to absorption, phosphatase enzymes within enterocytes catalyze the hydrolysis of FAD and FMN, releasing free riboflavin. Absorption occurs mainly in the small intestine through a carrier-mediated mechanism, where the riboflavin transporter RFVT3 facilitates uptake. This process is enhanced when riboflavin is consumed with food[29].
- **Distribution:** Following absorption, riboflavin is transported to both central and peripheral tissues and is capable of crossing the blood-brain barrier. Systemic distribution is mediated by the transporter RFVT2, encoded by SLC52A2, while the ATP-binding cassette transporter ABCG2 regulates its transfer into breast milk, cerebrospinal fluid, semen, and bile[29].
- **Metabolism**: Riboflavin undergoes enzymatic transformation into its biologically active coenzyme forms within the cytoplasm of various tissues, including the liver, heart, and kidneys. This process begins with flavokinase catalyzing the phosphorylation of riboflavin to flavin mononucleotide (FMN), which is then converted into flavin adenine dinucleotide (FAD) by FMN adenylyl transferase (FMNAT)[30].
- **Elimination**: The elimination of riboflavin occurs primarily through renal excretion, with an average half-life of approximately one hour. The majority of riboflavin is excreted unchanged in the urine, while less than 1% is eliminated through bile[30].

2.3. Pharmacodynamics

Riboflavin (vitamin B2) is a water-soluble micronutrient essential for human health. It plays a crucial role in energy metabolism by facilitating the breakdown of fats, carbohydrates, and proteins. Beyond its metabolic functions, riboflavin is integral to red blood cell production, respiratory processes, and immune response through antibody formation. It also contributes to cellular growth, reproductive health, and the maintenance of skin, nails, and hair. Additionally, riboflavin supports thyroid function and may aid in preventing or managing specific ocular conditions, including certain types of cataracts[31&32].

2.4. Mechanism of Action

Riboflavin is essential for macronutrient metabolism and the biosynthesis of specific B-complex vitamins. Its biological activity is mediated through its cofactors, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), which facilitate redox reactions by functioning as electron carriers. A key role of riboflavin in these reactions occurs within the electron transport chain, where its deficiency can impair fundamental metabolic pathways. FAD is required for the conversion of tryptophan to niacin, while FMN is necessary for the activation of vitamin B6 (pyridoxine) into pyridoxal 5′-phosphate. Additionally, riboflavin exhibits antioxidant properties by regenerating glutathione, a crucial molecule for neutralizing free radicals. It also plays an indispensable role in growth, development-particularly during fetal stages–reproductive health, and lactation[31&32].

Recent advancements in high-performance liquid chromatography (HPLC) techniques have significantly enhanced the sensitivity, specificity, and capability to analyze complex food samples for riboflavin (vitamin B2). Reversed-phase HPLC remains the preferred method, utilizing carefully optimized mobile phases–typically a combination of aqueous-organic solvents and buffering agents–to improve analyte retention and resolution. Given riboflavin's natural fluorescence, fluorescence detection is widely employed, offering high sensitivity and low detection limits. Improved sample preparation methods, such as enzymatic hydrolysis and solid-phase extraction, enhance recovery rates and minimize matrix interference, ensuring precise quantification across various food products, including dairy, grains, and fortified beverages. These methodological refinements reinforce the critical role of HPLC in nutritional analysis, facilitating accurate riboflavin monitoring for quality control and regulatory compliance (Table 4).

Table 4: HPLC method for the analysis of riboflavin (B2) in food samples.

Sample	Column	Mobile phase	Detector	Ref
Cabbage, Beets, Cauliflower, Lettuce	C_{18}	0.04 mol L^{-1} potassium dihydrogen phosphate buffer (pH 7): ACN (75:25, v/v)	UV (263 nm)	33
Mushrooms	C_{18} (150 × 4.6 mm i.d., 5 μm)	Buffer: methanol (80:20, v/v), pH 3.0	UV (B1: 245 nm, B2: 268 nm)	34
Frozen Fruits	C_{18} Hypersil GOLD aQ (150 × 2.1 mm, 3 μm)	(A) 2 M ammonium formate (5 mL), formic acid (1 mL); (B) methanol (995 mL), 2 M ammonium formate (5 mL), formic acid (1 mL)	LC-MS	35
Yeast	C_{18} column (250 × 4.6 mm, 5 μm)	(A) 0.05 M ammonium acetate: methanol (99:1, v/v); (B) water: methanol (50:50, v/v)	PDA	36
Fermented Soy Products (China)	C_{18}, Phenomenex (250 mm, 5 μm)	(A) Sodium acetate buffer (5 mM, pH 4.9); (B) Methanol (35%)	Fluorescence detector (λEx 462 nm, λEm 522 nm)	36
Okra Pods	RP (Agilent ZORBAX C_{18}, 250 × 4.6 mm, 5 μm)	(A: Methanol, B: 0.023 M H_3PO_4, pH 3.54)	UV detector (270 nm)	37
Five Wild Edible Fruits (Northeastern India)	C_{18} Hypersil (150 × 2.1 mm, 3 μm)	(A) Water (995 mL), 2 M ammonium formate (5 mL), formic acid (1 mL); (B) Methanol (995 mL), 2 M ammonium formate (5 mL), formic acid (1 mL)	PDA	36
Traditional Rice and Yams (Sri Lanka)	C_{18} column (150 × 4.6 mm)	ACN(50 mL), glacial acetic acid (10 mL), double-distilled water to 1000 mL	UV detector (210, 270, 280 nm)	38

3. Pyridoxal (B6)

Vitamin B6 (Fig. 1) is an essential biomolecule that plays a pivotal role in various metabolic, physiological, and developmental processes. It serves as a cofactor in more than 140 enzymatic reactions, including transamination, aldol cleavage, α-decarboxylation, racemization, and β- and γ-elimination, all of which are fundamental to amino acid metabolism. Beyond its enzymatic functions, vitamin B6 demonstrates significant antioxidant properties, comparable to those of carotenoids and tocopherols, by scavenging reactive oxygen species. It is also involved in carbohydrate and lipid metabolism, highlighting its broader significance in cellular energy regulation and metabolic homeostasis[39-41].

Vitamin B6, which includes pyridoxal, pyridoxine, and pyridoxamine, is essential for various metabolic functions. Measuring its levels in different food sources is fundamental for assessing dietary sufficiency and informing evidence-based nutritional guidelines (Table 5). High-performance liquid chromatography (HPLC) is extensively employed for the accurate quantification of pyridoxal in various food matrices. Research on cereal-based infant formulas indicates that specific pH conditions can diminish pyridoxal. Advanced HPLC techniques incorporating enzymatic conversion and fluorescence detection have been utilized to analyze individual vitamers and total vitamin B6 content in poultry products and plant-based foods. These methodologies are critical for refining food composition databases and enhancing understanding of B6 vitamer distribution across different dietary sources[42-44].

3.1. Biosynthesis

Three distinct pathways for vitamin B6 biosynthesis have been identified, though only a brief overview is provided here, as comprehensive reviews are available elsewhere. In eubacteria such as *Escherichia coli*, *de-novo* synthesis is mediated by the coordinated actions of pyridoxine biosynthesis proteins A and J (PdxA, EC 1.1.1.262, and PdxJ, EC 2.6.99.2). This process utilizes 4-phosphohydroxy-L-threonine (4HPT) and deoxyxylulose 5′-phosphate (DXP) to produce pyridoxine 5′-phosphate (PNP). Another *de-novo* pathway, conserved across bacteria, archaea, and eukaryotes, leads to the synthesis of pyridoxal 5′-phosphate (PLP). This pathway involves ribose 5′-phosphate or ribulose 5′-phosphate, in combination with glyceraldehyde 3′-phosphate or dihydroxyacetone phosphate, along with glutamine (Fig. 5). Pyridoxal 5'-phosphate (PLP) biosynthesis occurs through three distinct pathways: A salvage pathway and two *de-novo* pathways, one requiring 1-deoxy-D-xylulose 5'-phosphate (DXP) and the other functioning independently of DXP. The key molecular components involved in these pathways include pyridoxal 5'-phosphate (PLP) (5), 1-deoxy-D-xylulose 5'-phosphate (7), 4-(phosphohydroxy)-L-threonine (6), glyceraldehyde 3'-phosphate (11), dihydroxyacetone phosphate (12), ribose 5'-phosphate (9), ribulose 5'-phosphate (10), glutamine (13), pyridoxamine (PM) (3), pyridoxamine 5'-phosphate (PMP) (4), pyridoxine (PN) (1), pyridoxine 5'-phosphate (PNP) (8), and pyridoxal (PL) (2)[45&46].

3.2. Pharmacokinetics[47&48]

- **Absorption:** B vitamins are efficiently absorbed in the gastrointestinal tract, except in individuals with malabsorption disorders. Pyridoxine is primarily absorbed in the jejunum, reaching its peak plasma concentration (Cmax) approximately 5.5 hours after ingestion.
- **Distribution:** The biologically active form of pyridoxine, pyridoxal 5′-phosphate, constitutes at least 60% of circulating vitamin B6 and predominantly binds to albumin in the plasma.
- **Metabolism:** Pyridoxine acts as a prodrug and undergoes extensive hepatic metabolism. Its metabolic pathways involve both primary and secondary transformations, with partial interconversion back to pyridoxine. The principal metabolite is 4-pyridoxic acid.
- **Elimination:** The inactive metabolite, 4-pyridoxic acid, is primarily excreted in the urine.

3.3. Pharmacodynamics

Vitamin B6 (pyridoxine) is a water-soluble vitamin commonly used to prevent and treat deficiency and peripheral neuropathy, particularly in individuals undergoing isoniazid (INH) therapy. Research indicates that it reduces both systolic and diastolic blood pressure in patients with essential hypertension, a significant risk factor for atherosclerosis and coronary artery disease. Pyridoxine hydrochloride has been shown to inhibit platelet aggregation triggered by ADP or epinephrine and to influence lipid metabolism by lowering total cholesterol while increasing HDL levels. In its biologically active form, pyridoxal 5′-phosphate, vitamin B6 supports vascular endothelial function by reducing platelet-induced damage, a critical factor in the development of atherosclerosis[49&50].

Accurate quantification of vitamin B6 vitamers and their metabolites is crucial for evaluating B6 metabolism and nutritional status. Measurement challenges stem from the structural diversity of vitamers and the complexity of food matrices. Extensive reviews have examined existing analytical techniques. High-performance liquid chromatography (HPLC) is now the primary method, offering advantages over traditional microbiological and enzymatic assays. Different HPLC strategies are utilized to detect B6 vitamers in biological fluids with precision and reliability (Table 6). Nonexchange and paired-ion reversed-phase methods are widely utilized for analyzing vitamin B6 and its derivatives. The measurement of pyridoxal 5'-phosphate (PLP), the biologically active coenzyme form, in plasma and tissue extracts is efficiently conducted through a radioenzymatic assay, which facilitates high-throughput sample processing.

Vitamin B6 concentrations in food and biological samples are commonly determined using microbiological assays, with yeast growth-based methods, particularly those

employing *Saccharomyces uvarum* (ATCC 9080), being the most widely utilized. While variations in response among the three forms of vitamin B6 have been documented, these discrepancies are not observed in rapidly proliferating yeast cultures. Effective extraction of vitamin B6 vitamers is a critical prerequisite for all analytical techniques, with trichloroacetic acid (TCA) and perchloric acid recognized as efficient extractants.

A thorough understanding of vitamin B6's role in human nutrition necessitates detailed knowledge of its different forms and their concentrations in various dietary sources. Recent advancements in analytical chemistry, particularly high-performance liquid chromatography (HPLC), have enabled the precise quantification of pyridoxal, pyridoxine, and pyridoxamine vitamers even in complex food matrices such as cereal-based baby foods and plant-derived products. For instance, Hashim *et al.* demonstrated the application of HPLC–MS/MS for multi-form B6 analysis in fortified foods, offering enhanced selectivity and low detection limits suitable for regulatory and nutritional assessment purposes[51].

Similarly, recent reviews highlight the methodological shift from single-vitamin microbiological assays to multi-analyte chromatographic techniques as standard practice for B-complex quantification in food and supplements[52&53].

Table 5: Pyridoxal (vitamin B6) content of some foods[42-44].

Vegetables

Food	Vitamin B6 (mg/100 g)	Pyridoxine (%)	Pyridoxal (%)	Pyridoxamine (%)
Beans, lima, frozen	0.150	45	30	25
Cabbage, raw	0.160	61	31	8
Carrots, raw	0.150	75	19	6
Peas, green, raw	0.160	47	47	6
Potatoes, raw	0.250	68	25	7
Tomatoes, raw	0.100	38	49	13
Spinach, raw	0.280	35	52	13
Broccoli, raw	0.210	16	79	5
Cauliflower, raw	0.195	16	79	5
Corn, sweet	0.161	6	68	26

Fruits

Food	Vitamin B6 (mg/100 g)	Pyridoxine (%)	Pyridoxal (%)	Pyridoxamine (%)
Apples, Red Delicious	0.030	61	31	8
Apricots, raw	0.070	58	20	22
Apricots, dried	0.080	81	11	8
Avocados, raw	0.270	52	33	15
Bananas, raw	0.500	61	29	10
Oranges, raw	0.150	59	25	16
Peaches, canned	0.240	83	11	6
Raisins, seedless	0.313	—	—	—
Grapefruit, raw	0.070	—	—	—

Table 5: Continued.

Legumes & Nuts

Food	Vitamin B6 (mg/100 g)	Pyridoxine (%)	Pyridoxal (%)	Pyridoxamine (%)
Beans, white, raw	0.050	62	20	18
Beans, lima, canned	0.100	75	15	10
Lentils	0.600	79	14	7
Peanut butter	0.170	71	17	12
Peas, green, raw	0.160	69	17	14
Soybeans, dry, raw	0.810	44	44	12
Almonds, without skins, shelled	0.100	52	28	20
Pecans	0.183	71	12	17
Filberts	0.345	29	68	3
Walnuts	0.730	31	65	4

Cereals/Grains

Food	Vitamin B6 (mg/100 g)	Pyridoxine (%)	Pyridoxal (%)	Pyridoxamine (%)
Barley, pearled	0.224	52	42	6
Rice, brown	0.550	78	12	10
Rice, white, regular	0.170	64	19	17
Rye flour, light	0.090	64	14	22
Wheat, cereal, flakes	0.292	79	11	10
Wheat flour, whole	0.340	71	16	13
Wheat flour, all-purpose, white	0.060	54	24	21
Oatmeal, dry	0.140	12	49	39
Cornmeal, white and yellow	0.250	11	51	38
Bread, white	0.040	—	—	—
Bread, whole wheat	0.180	—	—	—

Meat/Poultry/Fish

Food	Vitamin B6 (mg/100 g)	Pyridoxine (%)	Pyridoxal (%)	Pyridoxamine (%)
Beef, raw	0.330	16	53	31
Chicken breast	0.683	7	74	19
Pork, ham, canned	0.320	8	84	8
Flounder fillet	0.170	7	71	22
Salmon, canned	0.300	2	9	89
Sardine, Pacific canned, oil	0.280	13	58	29
Tuna, canned	0.425	19	69	12
Halibut	0.430	—	—	—

Milk/Eggs/Cheese

Food	Vitamin B6 (mg/100 g)	Pyridoxine (%)	Pyridoxal (%)	Pyridoxamine (%)
Milk, cow, homogenized	0.040	3	76	21
Milk, human	0.010	0	50	50
Cheddar	0.080	4	8	88
Egg, whole	0.110	0	85	15

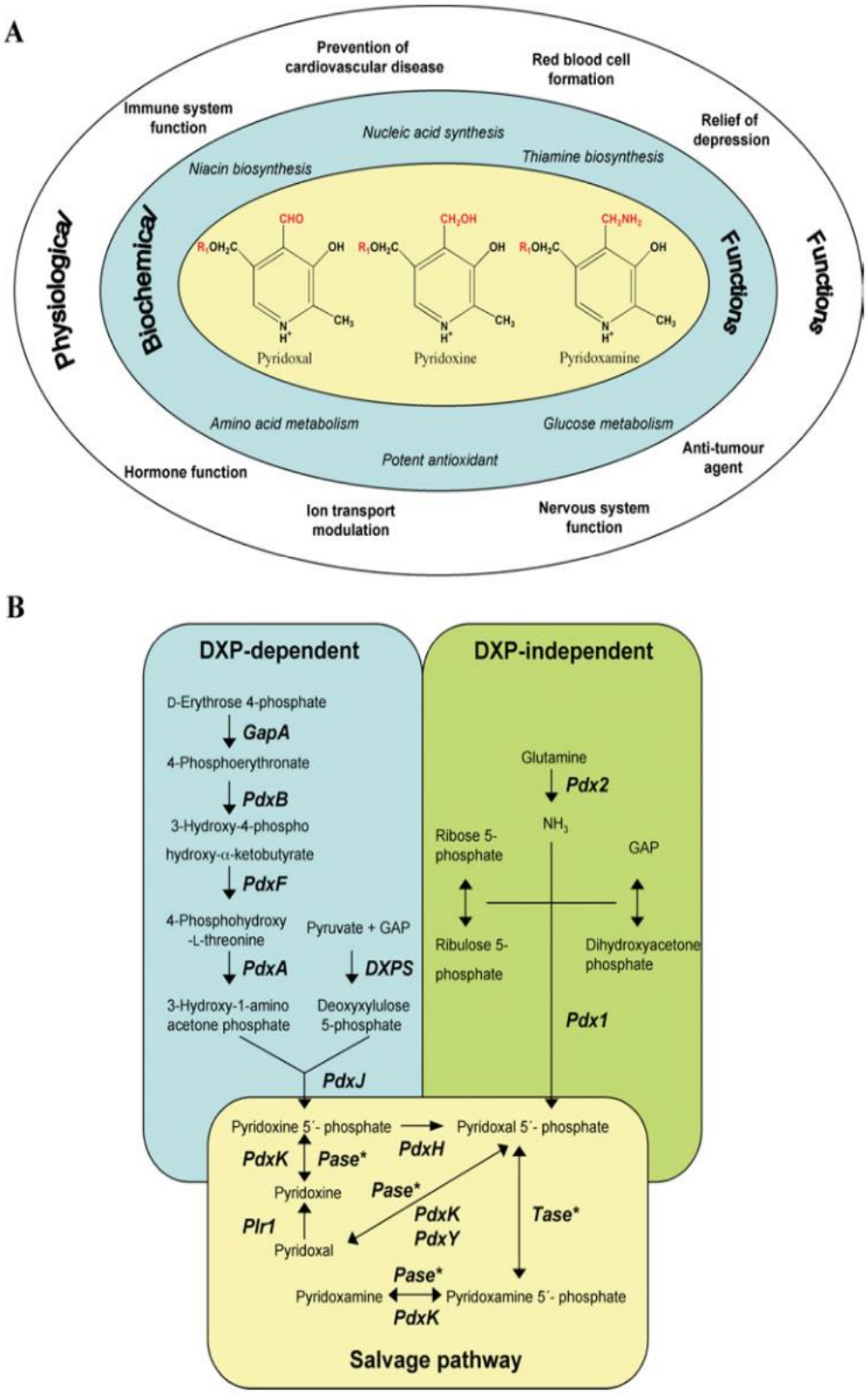

Fig. 5: Biosynthesis of pyridoxal (B6)[45].

Table 6: HPLC methods for the analysis of Pyridoxal (B6) in food samples.

Food sample	Column	Mobile phase	Detection	Ref.
Commonly consumed pulses in Bangladesh	C_{18} (250 × 4.6 mm, 5 μm)	Phosphate buffer (solvent A) and ACN (solvent B)	HPLC-DAD	54
Leafy vegetables (bottle gourd leaves, green amaranth, red amaranth, Indian spinach, bitter gourd)	C_{18} (ODS, 250 × 4.6 mm, 5 μm, Phenomenex)	Hexane sulfonic acid sodium salt, (pH 3.0), methanol (96:4, v/v)	UV (210 nm)	54
Cereal-based baby foods	X08-C_{18} (4.6 × 150 mm, 5 μm)	Buffer and methanol (70:30, v/v)	Fluorescence (λ_{Ex} 290 nm, λ_{Em} 395 nm)	44
Cow's milk	HSS T3 (100 × 2.1 mm, 1.7 μm)	A: 10 mM ammonium formate with 0.006% formic acid (pH 4.4); B: 10 mM ammonium formate with 0.7% formic acid	Fluorescence	55
Traditional yogurt (three cities in Iran)	C_{18} (250 × 4.6 mm, 5 μm)	10 mM phosphate buffer and CAN	UV (282 nm)	56
Supplemented foods and milk	C_{18} Waters ODS-2 (250 × 4.6 mm, 3 μm)	Phosphate buffer (solvent A) and methanol (solvent B)	Fluorescence	57
Manufactured food products	C_{18} BDS (100 × 4.6 mm, 3 μm)	A: 5.84 mM sodium hexane-1-sulfonate and ACN (95:5, v/v) (pH 2.5); B: 5.84 mM sodium hexane-1-sulfonate and ACN (50:50, v/v) (pH 2.5)	PDA (UV-DAD) and fluorescence (FLD)	58
Honey	C_{18} (250 × 4.6 mm, 5 μm)	Trifluoroacetic acid (0.025%, v/v) (A) and ACN (B)	UV (254 nm, 210 nm)	59
In-vitro and *ex-vitro* germinated chickpea	C_{18} (4.6 × 250 mm, 5 μm)	Buffer and methanol (96:4, v/v)	UV (210 nm)	60
Bee pollen	C_{18} (250 × 4.6 mm, 5 μm)	Phosphate buffer (pH 7.2, 0.228% $KH_2PO_4 \cdot 3H_2O$) and dimethylformamide (85:15, v/v)	Fluorescence (λ_{Ex} 368 nm, λ_{Em} 440 nm)	61
Raw and roasted nuts	C_{18} (250 × 4.6 mm, 5 μm)	Methanol (25%, v/v, for 5 min) and phosphate buffer (0.05 M, pH 7)	Fluorescence (λ_{Ex} 470 nm, λ_{Em} 525 nm)	62
Fruits and vegetables (Sindh, Pakistan)	C_{18} (4.6 × 250 mm, 5 μm)	50 mM K_2HPO_4 and methanol (70:30, v/v)	UV (254 nm)	63
Energy drinks	Phenomenex C_{18} (250 × 4.6 mm, 5 μm)	50 mM phosphate buffer (pH 3.0) and methanol (60:40, v/v)	UV (292 nm)	64

4. Cobalamin (B12)

Vitamin B12 (cobalamin) (Fig. 1) is an essential B-vitamin that plays a fundamental role in cellular metabolism, including DNA synthesis, methylation, and mitochondrial function. Although severe B12 deficiency, which manifests through hematologic and neurological symptoms, is relatively rare, subclinical deficiency affects an estimated 2.5% to 26% of the population, with its clinical significance remaining unclear. The risk of deficiency increases with age, making older adults particularly vulnerable. Major dietary sources include meat, dairy products, eggs, fish, and shellfish.

Absorption of vitamin B12 via the intrinsic factor-dependent pathway is limited to approximately 1.5 to 2.0 µg per meal, restricting bioavailability at higher intake levels. In healthy individuals, absorption efficiency varies depending on the source, with reported rates of approximately 42% from fish, 56%-89% from sheep meat, and 61%-66% from chicken. Traditional methods for quantifying vitamin B12 have encountered challenges related to sensitivity and selectivity. This study introduces a high-performance liquid chromatography-electrospray ionization mass spectrometry (HPLC-ESI-MS) approach, offering improved precision and sensitivity in determining vitamin B12 levels in food products and multivitamin-multimineral formulations[65-67].

Vitamin B12 is a crucial micronutrient primarily sourced from animal-based foods, many of which offer a high B12-to-calorie ratio. Shellfish, particularly clams and oysters, rank among the most concentrated sources, supplying substantial portions of the recommended daily intake in relatively small servings. Liver from beef and lamb is exceptionally rich in B12 and also provides other essential nutrients, including iron and vitamin A. Fatty fish such as salmon, mackerel, and tuna contribute significant amounts of B12 while also serving as valuable sources of omega-3 fatty acids. Dairy products, including milk, yogurt, and cheese, contain moderate levels of B12 and can be easily incorporated into daily diets. Eggs, where the yolk holds the highest concentration, offer a compact and versatile dietary source of B12 (Fig. 6 A and B). These foods collectively offer a high vitamin B12 content relative to caloric intake, enhancing the nutritional quality and density of the diet[68-70].

Fig. 6: A and B are popular vitamin B12 rich foods that provide more B12 per calorie[71].

4.1. Biosynthesis

Cobalamin biosynthesis occurs exclusively in prokaryotes through two distinct *de-novo* pathways, which differ in cobalt incorporation timing and oxygen dependence. The aerobic pathway, extensively studied in *Propionibacterium dentifricans*, contrasts with the anaerobic pathway, characterized in *Salmonella typhimurium*, *Bacillus megaterium*, and *Propionibacterium shermanii*. A key difference is cobalt chelation: The aerobic process involves the CobNST complex in hydrogenolytic *a,c*-diamide formation, while the anaerobic pathway in *S. typhimurium* utilizes CbiK and precorrin-2 for cobalt insertion. Oxygen is essential in the aerobic pathway, facilitating ring contraction, a process absent in anaerobic biosynthesis. Figure 7 illustrates tetrapyrrole biosynthetic pathways, indicating that δ-aminolevulinic acid (ALA) synthesis follows either the C4 or C5 route. Adenosylcobalamin formation occurs via *de-novo* synthesis or salvage mechanisms, with key biosynthetic enzymes originating from *P. dentifricans* (aerobic) or *S. typhimurium* (anaerobic)[72-74].

4.2. Pharmacokinetics[75&76]

- **Absorption:** Vitamin B12 is quickly absorbed after intramuscular (IM) or subcutaneous (SC) injection, with peak plasma levels appearing about one hour post-IM administration. When taken orally, B12 binds to intrinsic factor (IF) in the stomach and remains in this complex until reaching the terminal ileum. In the presence of adequate calcium, IF dissociates, allowing B12 to be absorbed into gastrointestinal mucosal cells via transcobalamin-mediated transport. A small portion of B12 can also cross the intestinal barrier through passive diffusion, a process that requires high doses (>1 mg). For oral doses below 3 mcg, peak plasma concentrations are delayed (8-12 hours) due to temporary retention in the lower ileum.
- **Distribution:** Cobalamin is distributed throughout the body, with the liver and bone marrow serving as primary storage sites.
- **Metabolism:** Dietary vitamin B12 (cyanocobalamin) initially binds to haptocorrin, a salivary protein that protects it from gastric degradation. In the duodenum, pancreatic proteases release B12 from haptocorrin and other binding proteins, enabling its association with IF. This IF-B12 complex is internalized in the terminal ileum via cubilin/AMN receptor-mediated endocytosis. Within enterocytes, lysosomal degradation of IF releases cobalamin into the cytoplasm. The transporter protein ABCC1 then facilitates the basolateral export of transcobalamin-bound B12 into the bloodstream. From there, it is transported through the portal vein to the liver before entering systemic circulation. The biologically active forms of vitamin B12 in the body include methylcobalamin and adenosylcobalamin.
- **Elimination:** Vitamin B12 is partially excreted in urine. Approximately 3-8 mcg of B12 is secreted daily into the gastrointestinal tract via bile, with nearly complete reabsorption occurring in individuals with adequate intrinsic factor, except for approximately 1 mcg. When plasma protein and hepatic binding capacities are exceeded, unbound B12 is rapidly eliminated in urine. The total body storage of B12 depends on the administered dose.

4.3. Pharmacodynamics[77&78]

- **Absorption:** A small fraction of cobalamin, approximately 1.2%, undergoes passive absorption across the intestinal wall, independent of intrinsic factor. At sufficiently high oral doses, this mechanism alone can correct vitamin B12 deficiency. When intrinsic factor is present in adequate amounts, it significantly enhances absorption efficiency. Parenteral administration bypasses the intestinal barrier, allowing direct entry into the bloodstream. Once in circulation, vitamin B12 binds to transcobalamin II (TCII), which facilitates its transport into target tissues via TCII receptors.
- **Cellular Effects and Mechanism of Action:** Cobalamin is essential for hematopoiesis and neurological function, serving as a cofactor for methionine synthase and methylmalonyl-CoA mutase. Methionine synthase catalyzes the conversion of homocysteine to methionine while simultaneously converting methyl-tetrahydrofolate (methyl-THF) into

tetrahydrofolate (THF), a precursor necessary for DNA synthesis. Methionine is further converted to S-adenosylmethionine, a key methyl donor involved in phospholipid synthesis, neurotransmitter production, and epigenetic regulation. Cobalamin deficiency disrupts this pathway, leading to megaloblastic anemia due to the "folate trap" mechanism, which impairs DNA replication.

In its adenosylcobalamin form, vitamin B12 acts as a cofactor for methylmalonyl-CoA mutase, an enzyme that converts methylmalonyl-CoA to succinyl-CoA. This reaction is vital for the metabolism of odd-chain fatty acids and branched-chain amino acids, highlighting cobalamin's role in energy production and cellular homeostasis.

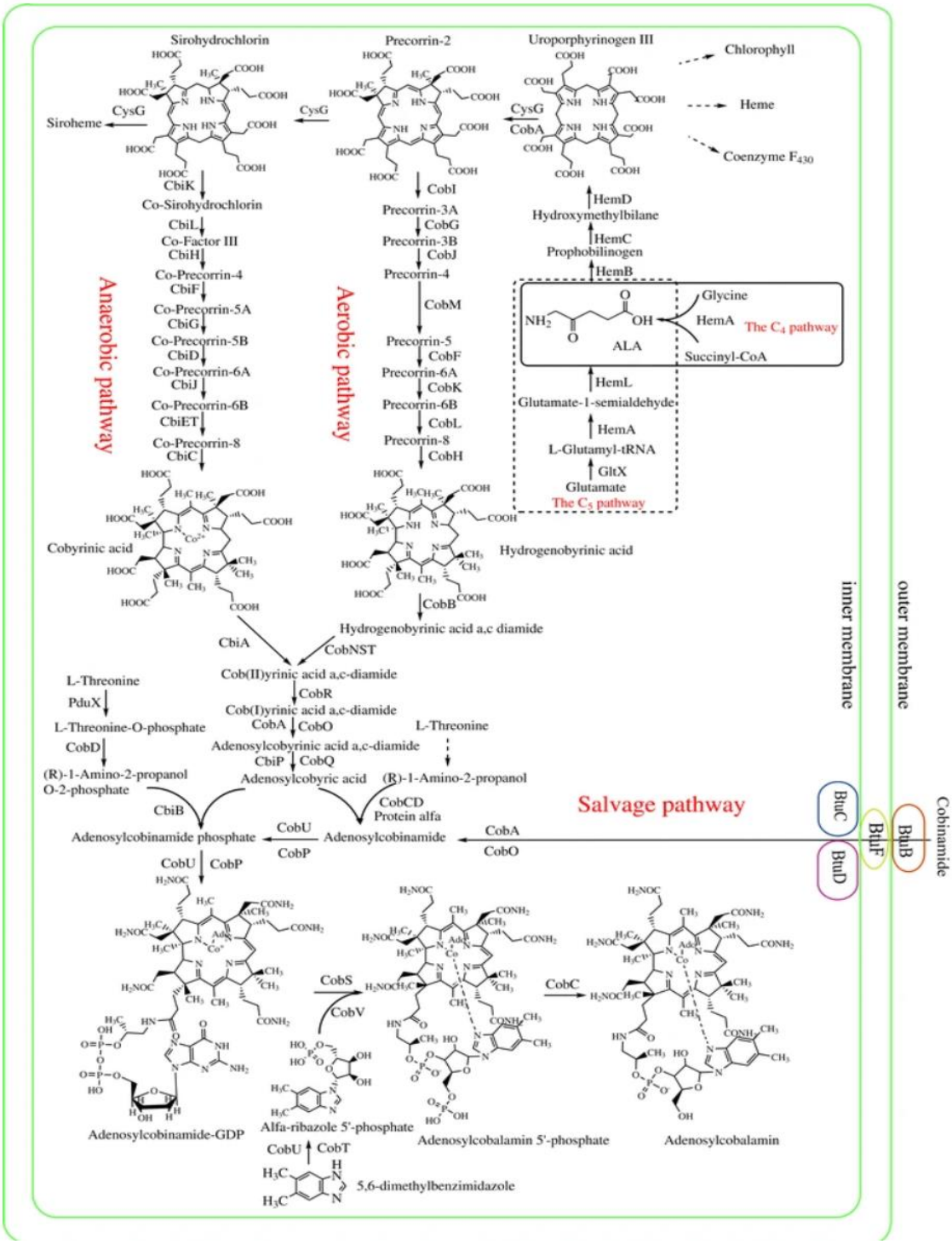

Fig. 7: Biosynthesis of cobalamin(B12)[79].

Recent advancements in high-performance liquid chromatography (HPLC) have substantially enhanced the accuracy and sensitivity of cobalamin (vitamin B12) quantification in food matrices (Table 7). The effectiveness of reversed-phase HPLC coupled with mass spectrometry (HPLC-MS) for detecting bioactive B12 forms in beef, offering valuable insights into its bioavailability in meat products. Many of these methodologies utilize pre-column derivatization to improve detection accuracy, particularly for trace B12 concentrations. These developments reinforce HPLC as a fundamental tool for standardizing B12 analysis across various food products and nutritional sources.

Table 7: HPLC methods for the analysis of cobalamin(B12) in food samples.

Food sample	Column	Mobile phase	Detection	Ref.
Energy drinks, multivitamin pills	C_{18} (250 × 4.6 mm i.d., 5 μm)	Gradient; phosphate buffer (pH 3.0)–ACN	UV at 210 nm	80
Premixes	C_{18} AQ (150 × 3.0 mm, Hichrom, UK)	Gradient; A: water + 0.025% trifluoroacetic acid (TFA), pH 2.6; B: ACN	UV at 361 nm	81
Milk-based products	C_{18} HD (125 × 3.0 mm)	Gradient; A: 1000 mL water + 250 μL TFA, pH 2.6; B: 1000 mL ACN	UV at 361 nm	82
Ripened cheese	C_{18} UHPLC® (50 × 2.1 mm, 1.7 μm)	Gradient; 5 mM ammonium formate in water (A) and ACN (B)	LC-MS/MS	83
Nutraceutical products	C_{18} (250 × 4.6 mm, 5 μm)	Gradient; methanol and buffer (30:70), pH 4.2	UV at 254 nm	84
Berries and seeds	Superdex 200 10/300GL	Gradient; 10 mM ammonium acetate buffer (pH 7.4)	μ-HPLC-ESI-MS	85
Suaeda vermiculata	ODS-C_{18} (250 × 4.6 mm, 5 μm)	Methanol: water (65:35, v/v)	UV (200–400 nm)	86
Dried pineapple (Ananas comosus), dairy rasgulla	C_{18} (4.6 × 150 mm, 3.5 μm)	H_3PO_4–ACN(1:60, v/v)	UV at 254 nm	87

Conclusion

High-Performance Liquid Chromatography (HPLC) has emerged as an indispensable analytical tool for the detection and quantification of water-soluble vitamins, including thiamine (B1), riboflavin (B2), pyridoxal (B6), and cobalamin (B12). This review highlights the advancements in HPLC methodologies, which have significantly improved sensitivity, specificity, and robustness over the past two decades. Innovations in chromatographic separation, enhanced detectors, and sample preparation techniques have addressed challenges such as matrix interferences and analyte instability, ensuring more accurate and reproducible results. The ability of HPLC to handle diverse and complex food matrices makes it a vital method for ensuring quality and safety standards in food and pharmaceutical industries. Continued optimization of these techniques will meet the increasing demand for reliable vitamin analysis, particularly as dietary patterns evolve, and fortified products proliferate. This progress underscores HPLC's critical role in advancing nutritional science and public health.

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مراجعة علمية نقدية للتطورات الحديثة في الكروماتوغرافيا السائلة عالية الأداء (HPLC) لتحليل الفيتامينات الذائبة في الماء

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تتناول هذه المراجعة التطور في استخدام تقنية الكروماتوغرافيا السائلة عالية الأداء (HPLC) للكشف عن الفيتامينات الذائبة في الماء مثل الثيامين (B1) ، الريبوفلافين (B2) ، البيريدوكسال (B6) ، والكوبالامين (B12) خلال الفترة من عام ٢٠٠٠ حتى عام ٢٠٢٤. تُعد هذه الفيتامينات عناصر أساسية في عمليات الأيض وتوليد الطاقة والدفاع المضاد للأكسدة ، مما يجعل تقديرها بدقة أمرًا ضروريًا في الصناعات الغذائية والدوائية. وبسبب حساسيتها العالية تجاه العوامل البيئية مثل الحرارة والضوء والأكسجين ، برزت الحاجة إلى طرق تحليلية دقيقة وموثوقة. أثبتت تقنية HPLC تفوقها بفضل حساسيتها العالية وقدرتها على تحليل المكونات المعقدة في الأغذية. وتوضح الدراسة التحسينات الكبيرة في تقنيات الفصل الكروماتوغرافي وأنظمة الكشف التي أدت إلى زيادة الدقة وقابلية التكرار والاعتمادية في تقدير الفيتامينات. كما تناولت التطورات في أجهزة الكشف وتقنيات التحضير المسبق للعينات التي أسهمت في تقليل تأثير المكونات المصفوفية وتحسين حدود الكشف. وتؤكد هذه المراجعة على أهمية استمرار تطوير تقنيات HPLC لتلبية الطلب المتزايد على تحليل الفيتامينات بدقة عالية وضمان جودة وسلامة المنتجات الغذائية والدوائية.