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Simultaneous quantification of B-complex vitamins in tablet dosage form by ultraviolet spectrophotometry using the absorption factor method

Effendy De Lux Putra[1*], Siti Morin Sinaga[1], and M. Deddy Harfiansyah[1]
[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia.

[*]Corresponding author's e-mail: effendy@usu.ac.id

ABSTRACT
A novel ultraviolet (UV) spectrophotometric method using the absorption factor approach was developed for the simultaneous quantification of B-complex vitamins (cobalamin, pyridoxine, riboflavin, niacinamide, and thiamine) in tablet dosage forms. The analysis was carried out using methanol as the solvent, with distinct wavelengths selected for each vitamin: 361 nm for cobalamin, 291 nm for pyridoxine, 269 nm for riboflavin, 262 nm for niacinamide, and 239 nm for thiamine. The method exhibited excellent linearity with correlation coefficients (r) of 0.9999 (cobalamin), 0.9988 (pyridoxine), 0.9997 (riboflavin), 0.9999 (niacinamide), and 0.9998 (thiamine), all of which met the ICH requirement of r ≥ 0.995. Precision, expressed as %RSD, was 0.7277% for cobalamin, 0.7192% for pyridoxine, 0.7290% for riboflavin, 0.7330% for niacinamide, and 0.7287% for thiamine, satisfying the ICH criterion of %RSD < 2%. Accuracy based on recovery was 100.0851% (cobalamin), 100.3548% (pyridoxine), 100.3322% (riboflavin), 100.7838% (niacinamide), and 100.4271% (thiamine), which fall within the ICH acceptable limits of 98–102%. The method also demonstrated adequate sensitivity with LOD values between 0.4862–0.9849 µg/mL and LOQ values of 1.4732–2.9847 µg/mL. This technique offers a straightforward, precise, and effective method for the simultaneous quantification of B-complex vitamins in tablet formulations. The method satisfies all ICH validation criteria and shows minimal interference from excipients, making it a reliable tool for quality control in pharmaceutical analysis.

Keywords:
Simultaneous quantification; B-complex vitamins; UV spectrophotometry; absorption factor.

Introduction

B-complex vitamins, comprising cobalamin (Vitamin B_{12}; $C_{63}H_{88}CoN_{14}O_{14}P$), pyridoxine (Vitamin B_6; $C_8H_{12}ClNO_3$), riboflavin (Vitamin B_2; $C_{17}H_{20}N_4O_6$), niacinamide or nicotinamide (Vitamin B_3; $C_6H_6N_2O$), and thiamine (Vitamin B_1; $C_{12}H_{17}ClN_4OS{\cdot}HCl$), are essential water-soluble micronutrients widely used in multivitamin tablet formulations (USP, 2015). These vitamins play critical physiological roles, making accurate quantification in combined dosage forms an important aspect of pharmaceutical quality control (Hanna et al., 2022). However, simultaneous determination of B-complex vitamins presents analytical challenges due to significant overlap in their UV absorption spectra. Although advanced analytical platforms such as HPLC (Anwar et al., 2024; Mia et al., 2024; Saini et al., 2021; Maritha & Labasy, 2018; Naz et al., 2016) including RPLC and HILIC modes (Chutkowski et al., 2022), TLC-densitometry (Żandarek et al., 2023), voltammetry (Moustafa et al., 2022), PPEC and HPTLC (Polak & Pajurek, 2021), mass spectrometry-based detection (Porter & Lodge, 2021), and even FTIR fingerprinting are capable of resolving multivitamin mixtures with high selectivity (Nugrahani & Kartini, 2016), they often demand expensive equipment, skilled operators, and more laborious sample preparation, which limits their feasibility for routine quality control in many laboratories. UV spectrophotometry

remains an attractive alternative due to its simplicity and cost-effectiveness, but requires appropriate mathematical approaches to overcome spectral interference.

A wide range of analytical techniques has been employed for the determination of B-complex vitamins, including cobalamin, pyridoxine, riboflavin, niacinamide, and thiamine, either as individual components or in combination within multivitamin formulations. Among them, ultraviolet (UV) spectrophotometry has emerged as a preferred method due to its simplicity, rapid operation, and cost-effectiveness (Sinaga et al., 2025; Harfiansyah et al., 2024; Anwar et al., 2024; Demirkaya et al., 2022; Nasution et al., 2018; Al-Sammaraee & Al-Sammaraee, 2017; Chotimah et al., 2015; Hegazy et al., 2015). The absorption factor (AF) method is a spectrophotometric approach that mathematically corrects the absorbance contribution of interfering components using a constant absorbance ratio between two selected wavelengths. By employing this ratio, spectral overlap can be resolved without derivatization or chromatographic separation, allowing simultaneous quantification of multiple analytes within a single mixture. Previous research has validated the applicability of the AF approach for various multicomponent pharmaceutical matrices (Sinaga et al., 2022; Chavada et al., 2018; Abdelwahab & Mohamed, 2017); however, reports involving five B-complex vitamins with closely overlapping absorption maxima remain limited, indicating the necessity for further study.

In light of this gap, the novelty of the present work lies in applying and validating an AF-based UV spectrophotometric technique for the simultaneous determination of five B-complex vitamins in tablet dosage form. This method effectively addresses spectral interference through a straightforward mathematical correction while offering a cost-efficient, separation-free workflow that complies with ICH validation requirements. Consequently, it provides a practical analytical alternative for routine quality control of multivitamin formulations.

Methods

Materials

A Shimadzu UV-1800 UV-Visible spectrophotometer, operated with UV Probe software version 2.42, pharmaceutical-grade standards of cobalamin, pyridoxine, riboflavin, niacinamide, and thiamine were provided by the Indonesian Food and Drug Authority, commercially available Nutrimax B Complex tablets (Suryaprana Nutrisindo, Indonesia), each containing 50 mcg cobalamin, 50 mg pyridoxine, 20 mg riboflavin, 50 mg niacinamide, and 50 mg thiamine, were purchased from a licensed local pharmacy. The solvent used was methanol (Merck) because it is a solvent commonly used in pharmaceutical analysis (Beckett & Stenlake, 1988), a UV solvent with high spectral transparency (Skoog et al., 2017), and has been reported to produce stable absorbance signals in UV measurements (Nasution et al., 2018).

Procedures

Preparation of Standard Solution

Primary stock solutions of cobalamin, pyridoxine, riboflavin, niacinamide, and thiamine were individually prepared at a concentration of 1000 µg/mL by accurately weighing 25 mg of each vitamin and dissolving them in methanol in separate 25 mL volumetric flasks. Subsequently, working standard solutions with a concentration of 100 µg/mL were obtained by transferring 2.5 mL of each primary solution into individual 25 mL volumetric flasks and diluting to volume with methanol.

Selection of Analytical Wavelength

To determine the optimal analytical wavelengths, a series of dilutions was prepared from the working standard solutions, yielding concentration ranges of 15–35 µg/mL for cobalamin, 7.5–15.5 µg/mL for pyridoxine, 16–28 µg/mL for riboflavin, 6–30 µg/mL for niacinamide, and 4–20 µg/mL for thiamine. Each solution was scanned over the wavelength range of 200–400 nm using

a UV-Visible spectrophotometer. The absorbance values obtained at different concentrations were used to identify the most suitable wavelengths for analytical quantification.

Analysis of Tablet Dosage Form

A total of 20 tablets were accurately weighed and finely powdered using a mortar and pestle to achieve homogeneity. Each tablet contained 50 mcg of cobalamin, 50 mg of pyridoxine, 20 mg of riboflavin, 50 mg of niacinamide, and 50 mg of thiamine. An amount of powder equivalent to 50 mg of pyridoxine was weighed, and the corresponding quantities of the other B-complex vitamins were calculated based on this proportion. The weighed sample was transferred into a 50 mL volumetric flask, and methanol was added to reach the volume. The mixture was sonicated for 15 minutes to ensure complete dissolution. The resulting solution was filtered using Whatman filter paper No. 42, with the initial 10 mL of the filtrate discarded. The remaining filtrate was collected, subsequently, 2.5 mL of the clear filtrate was transferred into a 25 mL volumetric flask and diluted to volume with methanol. In this solution, 1.15 mL was pipetted into a 10 mL volumetric flask, followed by the addition of working standard solutions of cobalamin, riboflavin, niacinamide, and thiamine in volumes of 2.5 mL, 1.74 mL, 0.65 mL, and 0.05 mL, respectively. The volume was adjusted to the mark using methanol. These solutions were scanned in the wavelength range of 200–400 nm using a UV-Visible spectrophotometer. The final concentrations of cobalamin, riboflavin, niacinamide, thiamine, and pyridoxine in the test solution were determined to be 25 µg/mL, 22 µg/mL, 18 µg/mL, 11.5 µg/mL, and 12 µg/mL, respectively. Quantitative analysis was performed using the calibration curve linear regression equations developed for each vitamin.

Method Validation

The proposed analytical method was validated according to the International Council for Harmonisation (ICH) guidelines, evaluating parameters such as linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ) (Sinaga et al., 2022; Chavada et al., 2018; Abdelwahab & Mohamed, 2017; ICH, 2005).

Linearity

Linearity was assessed by plotting the absorbance values against corresponding concentrations and calculating the correlation coefficient (r), which reflects the strength of the linear relationship (Sinaga et al., 2022; Chavada et al., 2018; Abdelwahab & Mohamed, 2017; ICH, 2005).

Accuracy

Accuracy was determined through a standard addition approach by spiking known amounts of analytes into the sample matrix at three concentration levels representing 80%, 100%, and 120% of the nominal concentration (Sinaga et al., 2022; Chavada et al., 2018; Abdelwahab & Mohamed, 2017; ICH, 2005).

Precision

Precision was evaluated by calculating the relative standard deviation (RSD) of replicate measurements, with an acceptance criterion set at RSD < 2%, indicating good repeatability (Sinaga et al., 2022; Chavada et al., 2018; Abdelwahab & Mohamed, 2017; ICH, 2005).

LOD

The LOD was estimated using the formula: $LOD = 3.3 \times (\sigma/S)$, where σ represents the standard deviation of the response and S is the slope of the calibration curve (Sinaga et al., 2022; Chavada et al., 2018; Abdelwahab & Mohamed, 2017; ICH, 2005).

LOQ

The LOQ was calculated using the formula: LOQ = 10 × (σ/S), providing the lowest concentration at which the analyte can be reliably quantified with acceptable accuracy and precision (Sinaga et al., 2022; Chavada et al., 2018; Abdelwahab & Mohamed, 2017; ICH, 2005).

Results and Discussion

Selection of Analytical Wavelength

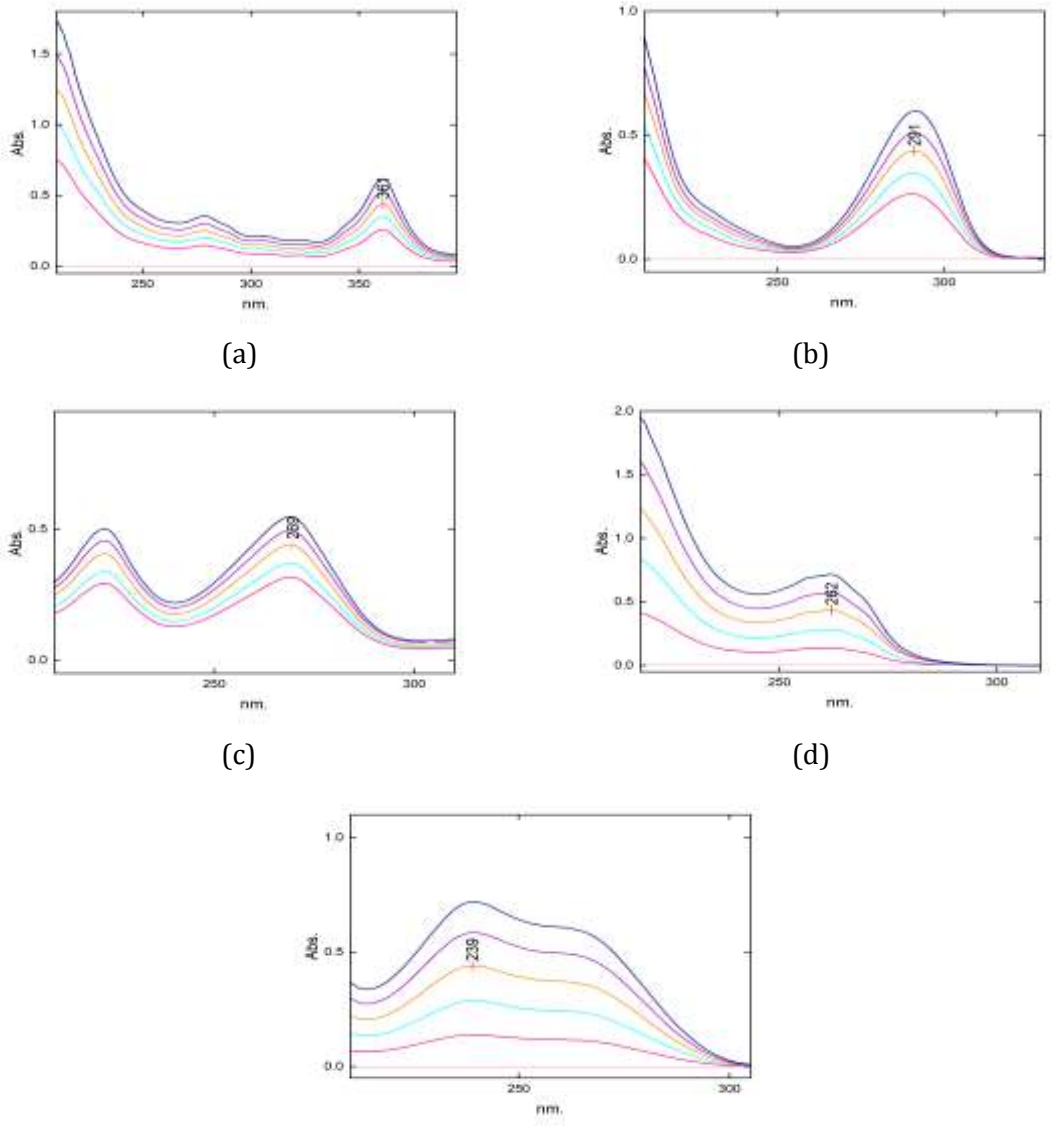

Figure 1. The absorption factor spectra (a) cobalamin wavelength 361 nm, (b) pyridoxine wavelength 291 nm, (c) riboflavin wavelength 269 nm, (d) niacinamide wavelength 262 nm, and (e) thiamine wavelength 239 nm

In the absorption factor method, wavelength selection plays a critical role in minimizing spectral interference among components (Figure 1). The first analytical wavelength (λ_1) was selected at 361 nm, where the absorbance of cobalamin is significant, and the spectral contributions from pyridoxine, riboflavin, niacinamide, and thiamine are negligible. The second wavelength (λ_2) was set at 291 nm, which corresponds to the maximum absorbance of pyridoxine

with minimal interference from riboflavin, niacinamide, and thiamine. The third wavelength (λ_3), 269 nm, was chosen based on the distinct absorbance of riboflavin, without overlapping signals from niacinamide and thiamine. Similarly, 262 nm (λ_4) was identified as the optimal wavelength for niacinamide determination, free from spectral interference by thiamine. Finally, thiamine was best detected at 239 nm (λ_5), where its absorbance is most prominent. The optimal wavelength for each analyte was determined based on its ability to produce a strong linear correlation between absorbance and concentration, as demonstrated by a correlation coefficient (r) approaching unity ($r \leq 1$) (Sinaga et al., 2022; Chavada et al., 2018; Abdelwahab & Mohamed, 2017; ICH, 2005). Wavelength selection was performed to minimize interference between vitamins. The spectrum in Figure 1 shows that cobalamin absorbs strongly at 361 nm, pyridoxine at 291 nm, riboflavin at 269 nm, niacinamide at 262 nm, and thiamine at 239 nm. The lack of significant overlap at these points supports the use of the absorption factor (AF) method for simultaneous quantification. AF-based separation proved effective without the need for derivatization or chromatographic fragmentation, making it more efficient than HPLC or previous UV-derivative methods. (Sinaga et al., 2022; Chavada et al., 2018; Abdelwahab & Mohamed, 2017).

Method Validation

The analytical procedure was validated in accordance with key performance parameters, including accuracy, linearity, precision, limit of detection (LOD), and limit of quantification (LOQ). A summary of the validation outcomes is presented in Table 1.

Table 1. Validation methods of cobalamin, pyridoxine, riboflavin, niacinamide, and thiamine in the absorption factor method

Parameter	**Cobalamin**	**Pyridoxine**	**Riboflavin**	Niacinamide	**Thiamine**
Linearity	0.9999	0.9988	0.9997	0.9999	0.9998
Accuracy (%)	100.0851	100.3548	100.3322	100.7838	100.4271
Precision (%RSD)	0.7277	0.7192	0.7290	0.7330	0.7287
LOD (µg/mL)	0.5727	0.9849	0.8512	0.6209	0.4862
LOQ (µg/mL)	1.7354	2.9847	2.5793	1.8814	1.4732

As shown in Table 1, the validation outcomes demonstrate that the developed method fulfills the criteria outlined by ICH guidelines for the simultaneous quantification of cobalamin, pyridoxine, riboflavin, niacinamide, and thiamine in tablet formulations. Recovery values obtained in this study were within the acceptable analytical validation criteria, ranging from 98–102%, which indicates no significant interference from tablet excipients. This suggests that the sample matrix did not affect absorbance at the selected wavelengths, demonstrating good method robustness for multivitamin formulations. In addition, the precision values remained below 2%, confirming that the procedure provides stable and reproducible results during repeated measurements (ICH, 2005). The compliance of all evaluated parameters confirms the reliability and suitability of this analytical approach. Previous investigations have also reported that ultraviolet spectrophotometry using the absorption factor method yields robust and acceptable validation results (Sinaga et al., 2022; Chavada et al., 2018; Abdelwahab & Mohamed, 2017).

Analysis Results of Tablet Dosage Form

As shown in Table 2 the quantified levels of cobalamin, pyridoxine, riboflavin, niacinamide, and thiamine in the tablet dosage form align with the labeled claims of B-complex vitamin content. Previous studies have also demonstrated the utility of similar methods (Sinaga et al., 2022; Chavada et al., 2018; Abdelwahab & Mohamed, 2017). In light of these findings, the absorption factor method proves to be a viable option for routine quality control of B-complex vitamin formulations containing multiple active components. When compared with other analytical approaches, the AF-based UV spectrophotometric method demonstrates several practical advantages. In routine quality control laboratories, UV-Vis instruments are more accessible and

economical than chromatographic systems, making the developed method particularly attractive for facilities with limited resources. While HPLC remains superior in terms of separation power and is often the reference technique for multivitamin analysis, it typically requires longer analysis time, expensive columns, high-purity solvents, and extensive sample preparation, which collectively increase operational costs. Conventional UV methods, such as derivative spectrophotometry or ratio spectroscopy, can also be applied to multicomponent mixtures; however, they may require additional spectral manipulation steps and optimization to avoid signal overlap. In contrast, the absorption factor approach resolves spectral interference mathematically without derivatization or separation, allowing rapid determination of five vitamins within a single measurement. The high recovery values obtained in this study further indicate that excipient interference is minimal, supporting the robustness of the AF approach for simultaneous multivitamin quantification. Overall, this work highlights that UV-AF can serve as a reliable, fast, and cost-effective alternative to chromatographic methods, especially for routine analysis and resource-limited settings.

Table 2. Quantitative determination results of cobalamin, pyridoxine, riboflavin, niacinamide, and thiamine in tablet dosage form using the absorption factor method

Component	Claim on the Label (mg)	The Content (mg)
Cobalamin	0.05	0.0500
Pyridoxine	50	50.3999
Riboflavin	20	19.9434
Niacinamide	50	50.6551
Thiamine	50	50.7060

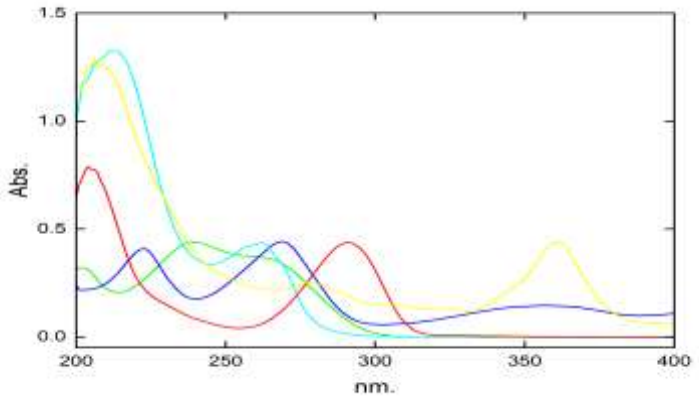

Figure 2. Overlain spectrum of cobalamin (line yellow), pyridoxine (line red), riboflavin (line blue), niacinamide (line cyan), and thiamine (line green)

Figure 2 illustrates that the spectral overlap of cobalamin at 25 µg/mL, pyridoxine at 11.5 µg/mL, riboflavin at 22 µg/mL, niacinamide at 18 µg/mL, and thiamine at 12 µg/mL, can be resolved with ultraviolet spectrophotometry using the absorption factor method.

Conclusion

The absorption factor method combined with ultraviolet spectrophotometry has been successfully applied for the simultaneous quantification of B-complex vitamins in tablet dosage forms, meeting the required validation parameters. This analytical approach is simple, accurate, sensitive, and precise, making it suitable for routine quality control of B-complex vitamins formulations.

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Conflict of interest

The authors report no conflicts of interest associated with this work.

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