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SIMULTANEOUS ANALYSIS OF THIAMINE AND PYRIDOXINE IN TABLETS USING THE UV SPECTROPHOTOMETRIC ABSORPTION FACTOR METHOD

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Abstract

Thiamine and pyridoxine are frequently combined in a single neurotropic tablet, yet their overlapping UV absorption spectra complicate simultaneous quantification, and a simplified absorption factor procedure dedicated to this specific binary combination has not been established. A UV spectrophotometric method based on the absorption factor approach was developed for the simultaneous quantification of thiamine and pyridoxine in tablet dosage forms using methanol as the solvent system. The proposed method was intended to minimize spectral overlap between both vitamins through mathematical absorbance correction at selected wavelengths. Thiamine showed maximum absorption at 239 nm, whereas pyridoxine exhibited its absorption maximum at 291 nm. Calibration curves demonstrated satisfactory linear relationships within the evaluated concentration ranges. Method validation was carried out according to ICH recommendations by assessing linearity, limit of detection, limit of quantification, accuracy, and precision. The percentage recovery values obtained for thiamine and pyridoxine were 98.48% and 100.43%, respectively, while the percentage relative standard deviation values indicated good repeatability of the analytical procedure. The developed method was successfully applied to commercial tablet preparations and produced assay results within acceptable pharmaceutical limits. The findings indicate that the proposed UV spectrophotometric absorption factor method is appropriate for routine quality control analysis of combined thiamine and pyridoxine tablet formulations.

Keywords: UV spectrophotometric; absorption factor; thiamine; pyridoxine

Abstrak

*Tiamin dan piridoksin sering dikombinasikan dalam satu tablet neurotropik, namun spektrum serapan UV keduanya yang saling tumpang tindih menyulitkan kuantifikasi secara simultan, dan prosedur faktor serapan sederhana yang khusus untuk kombinasi biner ini belum tersedia. Sebuah metode spektrofotometri UV berbasis pendekatan faktor serapan dikembangkan untuk kuantifikasi simultan tiamin dan piridoksin dalam bentuk sediaan tablet menggunakan metanol sebagai sistem pelarut. Metode yang diusulkan ini bertujuan untuk meminimalkan tumpang tindih spektrum antara kedua vitamin tersebut melalui koreksi absorbansi secara matematis pada panjang gelombang terpilih. Tiamin menunjukkan serapan maksimum pada 239 nm, sedangkan piridoksin menunjukkan serapan maksimum pada 291 nm. Kurva kalibrasi menunjukkan hubungan linear yang memuaskan dalam rentang konsentrasi yang dievaluasi. Validasi metode dilakukan sesuai dengan rekomendasi ICH dengan mengevaluasi linearitas, batas deteksi, batas kuantifikasi, akurasi, dan presisi. Nilai persentase perolehan kembali (*recovery*) yang diperoleh untuk tiamin dan piridoksin masing-masing adalah 98,48% dan 100,43%, sementara nilai persentase simpangan baku relatif menunjukkan keterulangan (*repeatability*) yang baik dari prosedur analisis tersebut. Metode yang dikembangkan berhasil diterapkan pada sediaan tablet komersial dan menghasilkan data kadar yang berada dalam batas farmasetis yang dapat diterima. Hasil penelitian menunjukkan bahwa metode*

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spektrofotometri UV dengan pendekatan faktor serapan yang diusulkan ini sesuai untuk analisis pengendalian mutu rutin pada formulasi tablet kombinasi tiamin dan piridoksin.

Kata kunci: spektrofotometri UV; faktor serapan; tiamin; piridoksin

INTRODUCTION

Thiamine and pyridoxine are water-soluble vitamins that play essential roles in maintaining normal metabolic and neurological functions. Both compounds are widely used in pharmaceutical preparations because they provide complementary therapeutic effects, particularly in the treatment of neuropathic disorders and vitamin deficiency conditions (Demirkaya et al., 2021). Thiamine functions as a coenzyme in carbohydrate metabolism and contributes to normal nerve impulse transmission. Deficiency of thiamine may cause neurological disorders, weakness, and impaired energy metabolism (Mrowicka et al., 2023). Pyridoxine plays a role in amino acid metabolism, neurotransmitter synthesis, and hemoglobin formation, making it beneficial in the management of neurological and metabolic disorders (Zhao et al., 2026).

The combination of thiamine and pyridoxine is commonly formulated in tablet dosage forms because combined therapy is considered more effective than the administration of a single vitamin alone. Therefore, a simple, fast, accurate and economical analysis method is needed to ensure the quality and content uniformity of both active ingredients in pharmaceutical products (Sinaga et al., 2025). Several analytical methods have been reported for the determination of thiamine and pyridoxine either individually or in combination, including high-performance liquid chromatography (HPLC) (Kamaris et al., 2025), thin-layer chromatography (TLC) densitometry (Żandarek et al., 2023), reversed-phase liquid chromatography (RP-LC) and hydrophilic interaction liquid chromatography (HILIC) (Chutkowski et al., 2022), voltammetric methods (Moustafa et al., 2022), pressurized planar electrochromatography (PPEC) and high-performance thin-layer chromatography (HPTLC) (Polak & Pajurek, 2021), liquid chromatography mass spectroscopy (LC-MS) (Porter & Lodge, 2021), fourier-transform infrared spectroscopy (FTIR) (Nasution et al., 2025), and ultraviolet-visible (UV-Vis) spectrophotometry (Demirkaya et al., 2021; Lohrdy et al., 2024; Harfiansyah et al., 2024; Putra et al., 2025; Sinaga et al., 2025). Although chromatographic techniques provide high sensitivity and selectivity, these methods generally require expensive instruments, more complicated sample preparation, and longer analysis time (Sinaga et al., 2025).

UV spectrophotometry has become an attractive alternative because it is simple, rapid, and suitable for routine analysis. However, simultaneous analysis using spectrophotometry is often limited by overlapping absorption spectra between compounds in a mixture. One approach that can be applied to overcome this limitation is the absorption factor (AF) method. This method enables simultaneous determination without prior separation or derivatization by using absorbance correction factors and simple mathematical calculations (Cerdà et al., 2022; Sinaga et al., 2022; Putra et al., 2025). The absorption factor method has been successfully applied in the simultaneous analysis of several pharmaceutical combinations and has shown acceptable validation performance in accordance with the International Council for Harmonization (ICH) guidelines, particularly for the parameters of linearity, limit of detection, limit of quantification accuracy, and precision (Panigrahi et al., 2026). In addition, this method is considered efficient for routine quality control analysis because it requires simpler instrumentation and lower operational costs compared with chromatographic methods (Anwar et al., 2024).

Several UV spectrophotometric approaches have been reported for the simultaneous determination of thiamine and pyridoxine, including chemometric partial least squares and principal component regression models (Demirkaya et al., 2021), soft independent modelling combined with partial least squares (López-de-Alba et al., 2006), and multivariate calibration in the presence of other analytes (Chotimah et al., 2015). Most of these methods rely on multivariate modelling and specialised software. The absorption factor method has previously been applied to five-component B-complex mixtures (Putra et al., 2025); however,

its application to the binary thiamine–pyridoxine combination as a dedicated, simplified procedure has not, to the best of our knowledge, been reported. The present study therefore develops a two-wavelength absorption factor method optimised specifically for this widely marketed binary combination.

Based on these considerations, the present study aimed to develop and validate a simple UV spectrophotometric absorption factor method for the simultaneous determination of thiamine and pyridoxine in tablet dosage forms.

Experimental Method

Materials

The study was carried out in three sequential stages consistent with its objective. First, the individual UV spectra of thiamine and pyridoxine were recorded to identify their absorption maxima and to characterise the spectral overlap responsible for mutual interference. Second, because pyridoxine absorbs at the thiamine maximum (239 nm) and thiamine absorbs at the pyridoxine maximum (291 nm), an absorption factor was derived for each analyte to correct these contributions mathematically. Third, the method was validated against ICH criteria and applied to commercial tablets.

All absorbance measurements were carried out using a Shimadzu UV-1800 UV-Visible spectrophotometer equipped with UV-Probe 2.42 software. Reference standards of thiamine and pyridoxine were obtained from the Indonesian Food and Drug Administration. Commercial Neurobion® tablets containing 100 mg thiamine and 200 mg pyridoxine were purchased from a local pharmacy. Methanol of analytical grade was used throughout the analysis.

Standard stock solutions of thiamine and pyridoxine were prepared separately in methanol at concentrations of 100 µg/mL. Working standard solutions were obtained by appropriate dilution of the stock solutions using the same solvent. The absorption spectra of thiamine and pyridoxine were recorded within the wavelength range of 200–400 nm to determine their maximum absorption wavelengths. Calibration curves were established using concentration ranges of 4–20 µg/mL for thiamine and 7.5–15.5 µg/mL for pyridoxine. The absorbance values obtained at the selected wavelengths were plotted against concentration to evaluate linearity.

Validation of the proposed method was performed according to ICH recommendations by evaluating linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision (Harfiansyah et al., 2024; Putra et al., 2025; Sinaga et al., 2025; Panigrahi et al., 2026).

Sample solutions containing thiamine (12 µg/mL) and pyridoxine (11.5 µg/mL) were prepared in methanol. The absorbance of each solution was measured at 239 nm and 291 nm. Since pyridoxine also showed absorbance at 239 nm, correction of spectral interference was carried out using the absorption factor equation. The concentration of thiamine was determined after subtracting the absorbance contribution of pyridoxine at the selected wavelength.

$$\text{Abs 239 nm (THI + PYR)} - \frac{\text{Abs 239 nm (PYR)}}{\text{Abs 291 nm (PYR)}} \times \text{Abs 291 nm (THI + PYR)}$$

Abs : Absorption value
THI : Thiamine
PYR : Pyridoxine

Results and Discussion

The maximum wavelengths were determined from the recorded absorption spectra of each vitamin. Based on Figure 1, thiamine exhibited a maximum absorbance at 239 nm, while pyridoxine showed its maximum wavelength at 291 nm. The R^2 values indicated good linear relationships between absorbance and concentration within the studied ranges, while the calibration curves are presented in Figure 2.

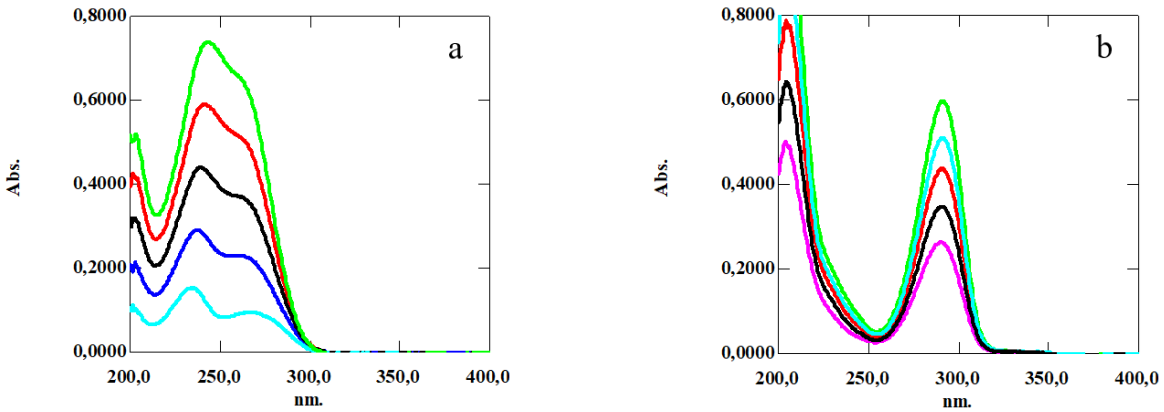


Figure 1. Overlaid UV Absorption Spectra of (a) Thiamine and (b) Pyridoxine at Various Concentrations, Showing Absorption Maxima at 239 nm and 291 nm, respectively

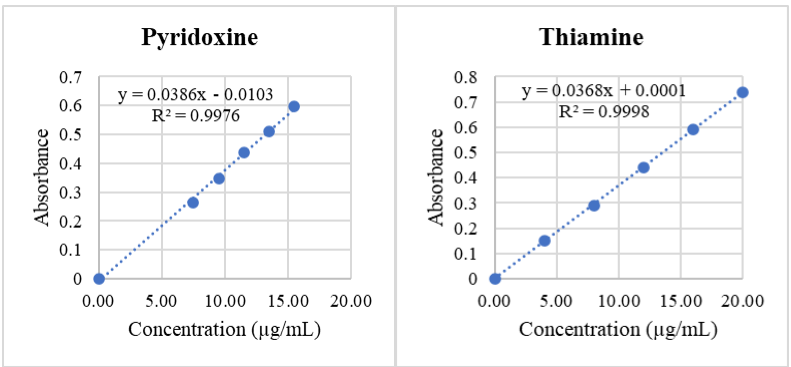


Figure 2. Calibration Curves of (a) Thiamine at 239 nm and (b) Pyridoxine at 291 nm

Based on Figure 1, thiamine and pyridoxine were measured at 239 nm and 291 nm, respectively. Pyridoxine also exhibited absorbance at 239 nm, as presented in Figure 3, which interfered with the determination of thiamine. Therefore, the absorption factor approach was applied to mathematically correct the absorbance contribution of pyridoxine at 239 nm. The correction process enabled selective quantification of thiamine without prior separation of the analytes.

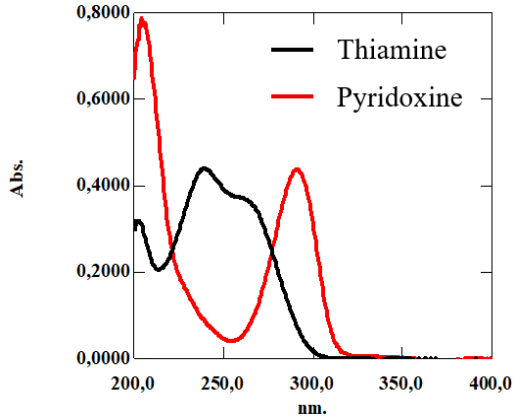


Figure 3. Overlain Spectra of Thiamine and Pyridoxine

The method was validated based on several validation parameters, including linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision are presented in Table 1.

Table 1. Validation Results of the AF Method for the Simultaneous Determination of Thiamine and Pyridoxine

Parameter	Thiamine	Pyridoxine
Linearity (R^2)	0.9998	0.9976
LOD (µg/mL)	0.35	0.98
LOQ (µg/mL)	1.07	2.98
Accuracy (%Recovery)	98.48	100.43
Precision (%RSD)	0.56	0.93

To confirm the absence of mutual interference, the absorbances of thiamine and pyridoxine measured individually were compared with those of their mixture, as shown in Table 2. The absorbance of pure pyridoxine at 239 nm (0.0888) and of pure thiamine at 291 nm (0.0751) were measured directly from single-component solutions. The measured absorbances of the mixture closely matched the sum of the absorbances of the individually measured components at both analytical wavelengths, with deviations below 0.5%, confirming additive behaviour and the absence of significant mutual interference, and thereby the selectivity of the method.

Table 2. Additivity Test of Thiamine and Pyridoxine Absorbances

Wavelength	Thiamine Alone	Pyridoxine Alone	Sum (Calculated)	Mixture (Measured)	Deviation
239 nm	0.4395	0.0888	0.5283	0.5267	0.30%
291 nm	0.0751	0.4366	0.5117	0.5105	0.24%

Method validation demonstrated satisfactory analytical performance for all evaluated parameters with requirements of ICH guidelines. The linearity values obtained for thiamine and pyridoxine indicated excellent correlation between concentration and absorbance response. The calculated LOD and LOQ values showed that the method possessed adequate sensitivity for detecting and quantifying both analytes at low

concentrations. Accuracy evaluation based on percentage recovery demonstrated that the proposed method produced acceptable recovery values for both vitamins. Precision assessment using percentage relative standard deviation also indicated good repeatability of the analytical measurements. These findings suggest that the developed method provides reliable analytical performance for routine pharmaceutical analysis.

The quantitative determination results of thiamine and pyridoxine in tablet dosage forms using the AF method are presented in Table 3

Table 3. Assay Results of Thiamine and Pyridoxine in Tablets Using the AF Method

Compound	The Content (mg)	Claim on the Label (mg)
Thiamine	95.97	100
Pyridoxine	194.99	200

The assay results presented in Table 3 obtained from commercial tablet preparations showed that the measured contents of thiamine and pyridoxine were consistent with the labeled claims and remained within acceptable pharmaceutical limits. Furthermore, the excipients present in the tablet formulation did not significantly interfere with the analysis, indicating satisfactory selectivity of the proposed spectrophotometric procedure. Compared with more sophisticated chromatographic techniques, the absorption factor spectrophotometric method offers several practical advantages, including simpler sample preparation, shorter analysis time, and lower operational cost. Therefore, the proposed method may serve as an alternative analytical procedure for routine quality control of combined vitamin tablet formulations.

The absorption factor method has been successfully applied to a range of overlapping drug mixtures. Table 4 compares the present method with reported absorption factor methods for other compounds. In every case the recoveries fell within the acceptable range (approximately 98–105%), confirming that the absorption factor approach is well established for resolving binary and ternary overlapping spectra without prior separation. The present study extends this validated approach to the thiamine–pyridoxine combination, obtaining recoveries (98.48% and 100.43%) consistent with those reported for other analytes.

Table 4. Absorption Factor Methods Reported for Other Compounds

Analytes (compounds)	Method	Recovery (%)	Reference
Metronidazole, Diloxanide, Mebeverine	Modified Absorption Factor	99.93–100.32	Abdelwahab & Mohamed (2017)
Betamethasone Valerate, Tazarotene	Absorption Factor	99.84–104.64	Mahmood et al. (2020)
Amlodipine Besylate, Valsartan	Absorption Factor	99.64–100.29	Sinaga et al. (2022)
Thiamine, Pyridoxine	Absorption Factor	98.48–100.43	This work

Recovery values are expressed as the range across the reported analytes and concentration levels.

The results obtained by the proposed method were also compared with those reported for thiamine and pyridoxine using other analytical techniques over (Table 5). The recoveries obtained in this work (98.48% and 100.43%) are consistent with the values reported for the same vitamins determined by high-performance liquid chromatography (Kamaris et al., 2025), chemometric UV methods (Demirkaya et al., 2021), and the area-under-the-curve technique (Harfiansyah et al., 2024), all of which lie within the acceptable range of 98–102%. This agreement confirms the reliability of the developed absorption factor method, which achieves comparable accuracy using only simple instrumentation.

Table 5. Other Reported Methods for Thiamine and Pyridoxine

Method	Instrumentation	Recovery (%)		Reference
		Thiamine	Pyridoxine	
Reversed-Phase HPLC	DAD/FLD	<100 ± 3	<100 ± 3	Kamaris et al. (2025)
Chemometrics (PLS/PCR)	UV-Vis	≤98.14	≤98.14	Demirkaya et al. (2021)
Area Under the Curve	UV-Vis	100.07	100.02	Harfiansyah et al. (2024)
Absorption Factor	UV-Vis	98.48	100.43	This work

Thiamine and pyridoxine, where a single value was reported for both vitamins, it is shown in both columns.

CONCLUSION

The present study successfully developed a UV spectrophotometric method based on the absorption factor approach for the simultaneous determination of thiamine and pyridoxine in tablet dosage forms. The proposed method demonstrated satisfactory validation performance in terms of linearity, accuracy, precision, and applicability to commercial pharmaceutical products. In addition, the method effectively minimized spectral interference through absorbance correction calculations without requiring prior separation procedures. Owing to its simplicity, rapid analysis, and cost-effectiveness, the method can be considered suitable for routine quality control analysis of thiamine and pyridoxine in combined tablet formulations.

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