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Simultaneous Determination of Vitamins B1, B2, B3, B6, and B12 in Tablet Forms Using the Area Under the Curve Ultraviolet Spectrophotometry Method

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ABSTRACT

This research focuses on creating the area under the curve (AUC) ultraviolet spectrophotometry method approach to assess the concentrations of vitamins B1, B2, B3, B6, and B12 in tablet formulations, bypassing the need for separation steps. The technique relies on calculating the AUC values across different concentrations within a specified wavelength range, using methanol as the solvent. Subsequently, the method was applied to evaluate the levels of these vitamins in the tablets. The analysis targeted specific wavelength ranges for each vitamin: 250-260 nm for B1, 279-289 nm for B2, 267-277 nm for B3, 312-322 nm for B6, and 382-392 nm for B12. The average recovery rates achieved were 100.07% for B1, 100.70% for B2, 100.52% for B3, 100.02% for B6, and 99.90% for B12 in the tablet samples. This method proved effective for the simultaneous analysis of vitamins B1, B2, B3, B6, and B12 in tablet forms and satisfied the necessary validation criteria.

Keywords: Vitamins B1, B2, B3, B6, B12, the area under the curve, ultraviolet spectrophotometry.

INTRODUCTION

The market offers supplements that often contain multiple active ingredients in a single formulation. Among these, vitamins play a crucial role (Sholekhudin, 2014), such as vitamins B1, B2, B3, B6, and B12 found in tablet form. These vitamins are essential for metabolic processes and energy production, functioning as coenzymes (Sumardjo, 2006; Tjay & Rahardja, 2007). Various techniques have been documented for quantifying vitamins B1, B2, B3, B6, and B12, either separately or together. These methods include spectrophotometry (Hegazy et al., 2015; Al-Sammaraee & Al-Sammaraee, 2017; Nasution et al., 2018), FTIR (Nugrahani & Kartini, 2016), HPTLC (Velmurugan et al., 2018; Polak & Pajurek, 2021), PPEC (Polak & Pajurek, 2021), HPLC (Sabaruddin et al., 2022), ion-pair chromatography (Diana, 2022), TLC-densitometry (Żandarek et al., 2023). The Area Under Curve (AUC) method in ultraviolet spectrophotometry allows for the concentration measurement of multi-component supplements without the need for prior derivatization or separation (Nasution et al., 2018; Bachri, 2019; Muchlisyam et al., 2019; Tarigan et al., 2023). However, there are currently no references to the use of AUC ultraviolet spectrophotometry for the simultaneous analysis of vitamins B1, B2, B3, B6, and B12 in tablet formulations. This study aims to establish a method using AUC ultraviolet spectrophotometry range to quantify the levels of these vitamins in tablet form.

MATERIALS & METHODS

1. Instruments and Materials

The study utilized a Shimadzu 1800 UV-visible spectrophotometer, which is equipped with UV Probe 2.42 software, pharmaceutical grades of vitamins B1, B2, B3, B6, and B12 were from the Indonesian Food and Drug Administration, methanol was obtained from Merck, and Nutrimax B Complex tablets, containing 50 mg of vitamin B1, 20 mg of vitamin B2, 50 mg of vitamin B3, 50 mg of vitamin B6, and 50 mcg of vitamin B12 (produced by Suryaprana Nutrisindo, Indonesia), were purchased from a local pharmacy.

2. Preparation of Standard Solution

Solutions of vitamins B1, B2, B3, B6, and B12 with a concentration of 1000 µg/ml were created by accurately weighing 25 mg of each vitamin into five distinct 25 ml volumetric flasks, using methanol as the solvent (First Standard Solution). To obtain a concentration of 100 µg/ml, 2.5 ml of the First Standard Solution was pipetted into five separate 25 ml volumetric flasks, again using methanol as the solvent (Second Standard Solution).

3. Selection of Analytical Wavelength

Dilutions were prepared from the Second Standard Solution to achieve the following concentrations: vitamin B1 (4-20 µg/ml), vitamin B2 (16-28 µg/ml), vitamin B3 (6-30 µg/ml), vitamin B6 (7.5-15.5 µg/ml), and vitamin B12 (15-35 µg/ml). These solutions were then scanned across a wavelength range of 200 to 400 nm. The AUC values for each spectrum at different concentrations were calculated to determine the optimal analytical wavelength.

4. Testing on Tablet Preparations

Twenty tablets were weighed and ground into a fine powder using a mortar and pestle until a uniform consistency was achieved. Each tablet contains 50 mg of vitamin B1, 20 mg of vitamin B2, 50 mg of vitamin B3, 50 mg of vitamin B6, and 50 mcg of vitamin B12. A total of 838.5 mg of the powder, which corresponds to 50 mg of vitamin B6, was then measured, and the amounts of vitamins B1, B2, B3, and B12 contained within it were calculated. This powdered sample was transferred to a 50 ml volumetric flask, and methanol was added to reach the mark. The mixture was sonicated for 15 minutes to ensure homogeneity. The solution was filtered with Whatman filter paper no. 42, discarding approximately 10 ml of the initial filtrate and collecting the remainder. A volume of 2.5 ml of the filtered solution was pipetted into a 25 ml volumetric flask, with methanol added to the mark. Then, 1.15 ml of the filtrate was pipetted into a 10 ml volumetric flask, and the Second Standard Solution of vitamins B1, B2, B3, and B12 was added in volumes of 0.05, 1.74, 0.65, and 2.5 ml respectively as an addition, followed by methanol to the mark. These solutions were then scanned in the wavelength range of 200 to 400 nm. Consequently, the concentrations of vitamins B1, B2, B3, B6, and B12 in the tablets were determined to be 12, 22, 18, 11.5, and 25 µg/ml, respectively. The final solution was analyzed using the proposed method, and quantification was performed by applying these values to the straight-line equation derived from the calibration curve.

5. Method Validation

This approach is validated in accordance with the ICH guidelines, focusing on parameters such as linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision (Nasution et al., 2018; Bachri, 2019; Muchlisyam et al., 2019; Tarigan et al., 2023).

5.1. Linearity

The correlation coefficient is calculated to assess linearity (Nasution et al., 2018; Bachri, 2019; Muchlisyam et al., 2019; Tarigan et al., 2023).

5.2.LOD

The limit of detection (LOD) is calculated using the following formula: LOD = 3.3 x (Standard Deviation /Slope) (Harmita, 2004).

5.3.LOQ

The limit of quantification (LOQ) is calculated using the following formula: LOQ = 10 x (Standard Deviation/Slope) (Harmita, 2004).

5.4.Accuracy

In the accuracy assessment, the standard addition method was employed, which involved preparing three concentrations of the sample analytes at specific levels of 80%, 100%, and 120% (Nasution et al., 2018; Bachri, 2019; Muchlisyam et al., 2019; Tarigan et al., 2023).

5.5. Precision

Precision is evaluated by calculating the relative standard deviation (RSD), with an acceptable threshold of less than 2% (Nasution et al., 2018; Bachri, 2019; Muchlisyam et al., 2019; Tarigan et al., 2023).

RESULT & DISCUSSION

1. Selection of Analytical Wavelength

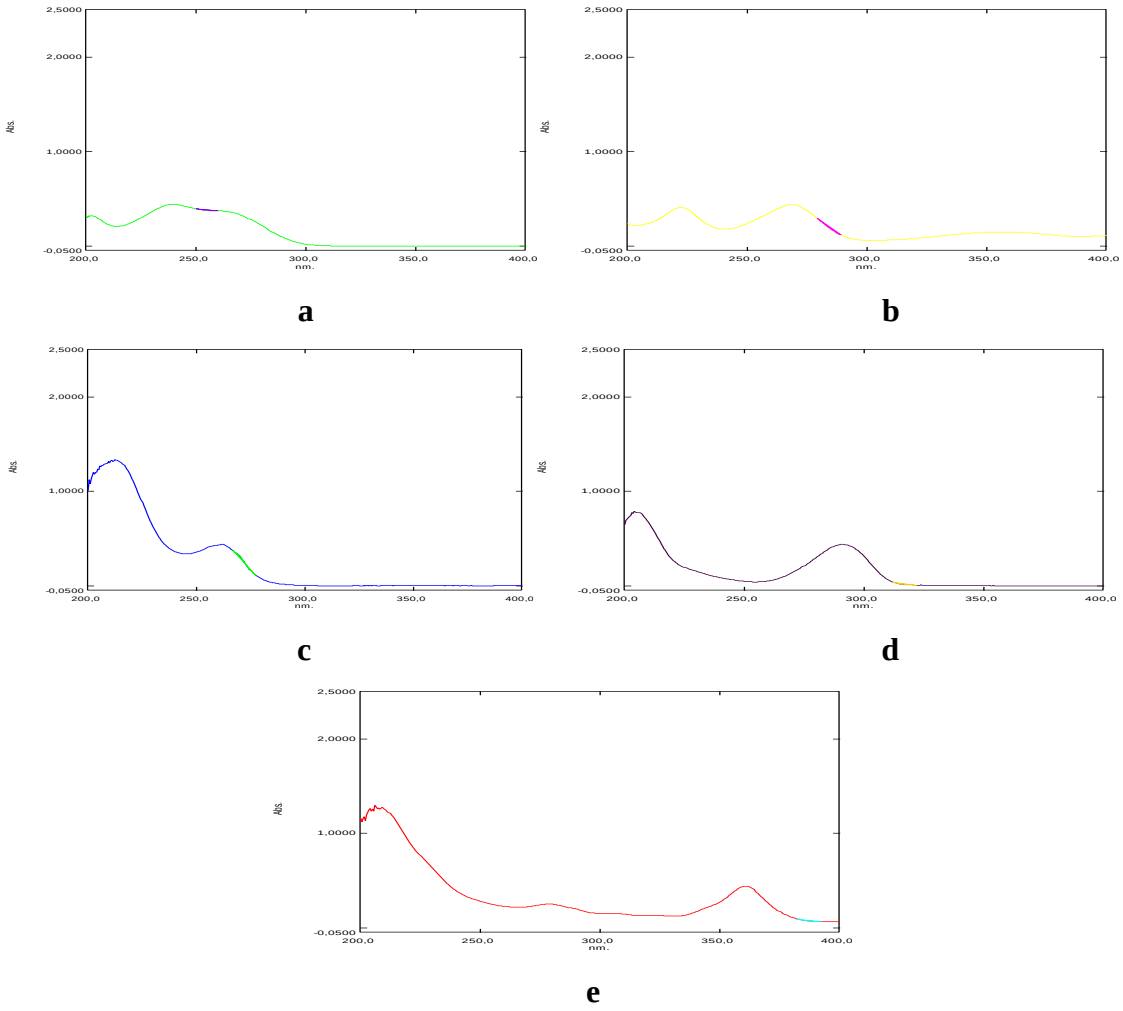

Figure 1. AUC (a) Vitamin B1 wavelength ranges of 250-260 nm, (b) Vitamin B2 wavelength ranges of 279-289 nm, (c) Vitamin B3 wavelength ranges of 267-277 nm, (d) Vitamin B6 wavelength ranges of 312-322 nm, and (e) Vitamin B12 wavelength ranges of 382-392 nm

The analytical wavelength was chosen by calculating the AUC values for each spectrum at varying concentrations throughout the absorption spectrum range. As shown in **Figure 1**, the absorption spectrum ranges for vitamins B1, B2, B3, B6, and B12 are identified at wavelengths of 250-260 nm, 279-289 nm, 267-277 nm, 312-322 nm, and 382-392 nm, respectively. This selected wavelength range demonstrates the strongest linear correlation between AUC values and concentration, as indicated by the correlation coefficient ($r \leq 1$) (Nasution et al., 2018; Bachri, 2019; Muchlisyam et al., 2019;

Tarigan et al., 2023). **Figure 1** also illustrates that there is no overlap in the AUC for the specified wavelength ranges of vitamins B1, B2, B3, B6, and B12, ensuring that they do not interfere with one another. Consequently, the area under the curve ultraviolet spectrophotometry method can effectively be applied to simultaneously quantify the content of multi-component supplements (Abdelwahab & Mohamed, 2017; Bachri, 2019; Muchlisyam et al., 2019; Tarigan et al., 2021; Tarigan et al., 2023).

2. Method Validation

The method was validated according to parameters such as linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision. The findings from the validation process are presented in **Table 1**.

Table 1. Method Validation for Vitamins B1, B2, B3, B6, and B12 Using the AUC Method

Parameters	Vitamin B1	Vitamin B2	Vitamin B3	Vitamin B6	Vitamin B12
Linearity	-0.9988	-0.9976	0.9989	-0.9954	-0.9991
LOD (µg/ml)	1.38	2.53	1.94	1.94	1.98
LOQ (µg/ml)	4.17	7.66	5.89	5.87	6.01
Accuracy (%)	100.07	100.70	100.52	100.02	99.90
Precision (%)	0.97	0.79	1.56	1.35	1.08

According to **Table 1**, this study demonstrates strong validation results for the simultaneous determination of vitamins B1, B2, B3, B6, and B12 in tablet form, as all validation parameters meet the ICH guidelines. This indicates that the methods conform to the necessary validation standards. Previous research has also reported favorable validation outcomes using the area under the curve ultraviolet spectrophotometry method (Abdelwahab & Mohamed, 2017; Bachri, 2019; Muchlisyam et al., 2019; Tarigan et al., 2021; Tarigan et al., 2023).

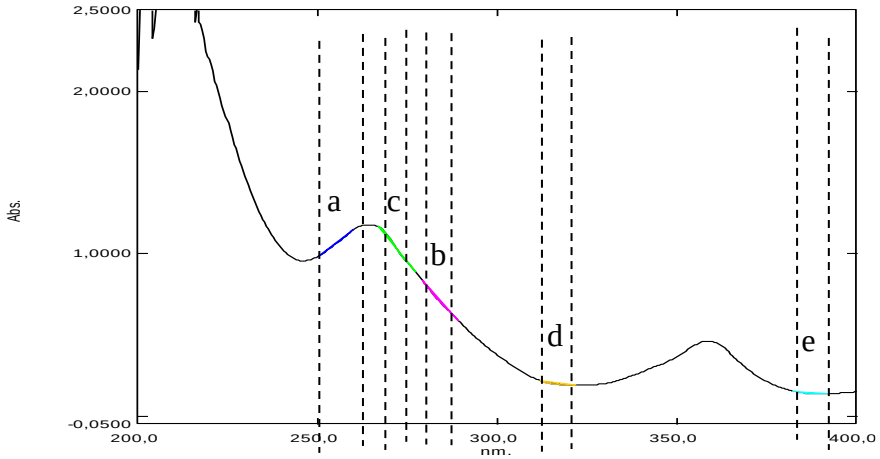

Figure 2. AUC Spectrum of Mixture Vitamins B1, B2, B3, B6, and B12 with a: Vitamin B1 (250-260 nm), b: Vitamin B2 (279-289 nm), c: Vitamin B3 (267-277 nm), d: Vitamin B6 (312-322 nm), e: Vitamin B12 (382-392 nm)

3. Test Results on Tablet Preparations

The results for the determination of vitamins B1, B2, B3, B6, and B12 in tablet form using this method are presented in **Table 2**.

Table 2. Results of the Concurrent Quantification of Vitamins B1, B2, B3, B6, and B12 in Tablet Form

Component	Claim on the Label (mg)	The Content (mg)
Vitamin B1	50	52.31
Vitamin B2	20	20.75
Vitamin B3	50	52.81
Vitamin B6	50	50.37
Vitamin B12	0.05	0.05

According to **Table 2**, this study indicates that the levels of vitamins B1, B2, B3, B6, and B12 in tablet form, as determined by the AUC method, align with the claims on the supplement labels. Furthermore, previous research (Abdelwahab & Mohamed, 2017; Bachri, 2019; Muchlisyam et al., 2019; Tarigan et al., 2021; Tarigan et al., 2023), supports the findings, suggesting that the proposed method holds promise for routine analysis of supplements, particularly those containing multiple components.

CONCLUSION

The area under the curve ultraviolet spectrophotometry method has been effectively utilized for the simultaneous quantification of vitamins B1, B2, B3, B6, and B12 in tablet formulations, meeting all validation criteria. This method is straightforward, accurate, sensitive, and precise, making it highly applicable in practice.

Declaration by Authors

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Conflict of Interest: The authors declare no conflict of interest.

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