Simultaneous Analysis Of Tablets Content Vitamin C And Zinc In Visible Spectrophotometry

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Abstract
Most drugs on the market contain multiple active ingredients, each of which is intended to enhance the drug's therapeutic effect and ease of administration. Determination of the levels of efficacious substances in drug preparations is essential for agencies that conduct drug determinations, such as the Food and Drug Supervisory Agency (BPOM) and the drug industry; therefore, a rapid and dependable analytical method, as well as relatively inexpensive and easily accessible tools and operational costs, are required. In practice, however, it can produce accurate and precise results. This research will be conducted by optimizing the solvent and simultaneously analyzing the levels of Vitamin C and Zinc in the tablet preparation without any separation step using visible spectrophotometry. Vitamin C and Zinc were found to be soluble in a mixture of methanol and water with a ratio of 50:50 after a single examination with the addition of the complexing agent dhitizon at a concentration of 0.1%, which produced a pink color. Calculation of Vitamin C and Zinc's linearity. The linearity value described is the correlation coefficient value for both Vitamin C and Zinc, which is very close to one, indicating an excellent relationship between the drug concentration and the absorbance value. This also suggests that absorbance will increase as concentration increases. The method validation requirements for linearity, accuracy, and precision as well as the limit of detection (LOD) and the limit of quantification (LOQ) were met by the analytical method validation with promising results.

Keywords: *Simultaneous Analysis, Vitamin C, Zinc and Spectrophotometry Visible.*

I. INTRODUCTION

Vitamin C is a type of vitamin that dissolves in water and is vital to human health. It protects the plasma lipids from free radical damage. It is essential for immune function, including the activity of leukocytes, phagocytosis, and chemotaxis, as well as the suppression of viral replication and production of interferon [1]. Zinc has long been known to play an essential role in the immune system and the body's resistance to various infections and diseases [2]. Zinc can act as an antioxidant and is involved in several important biochemical reactions in the body, which include protein synthesis, enzymatic functions and carbohydrate metabolism [3].Most drugs in circulation combine several active ingredients, each of which aims to increase the drug's therapeutic effect and ease of use. In marketing, quality inspection of a medicinal preparation is necessary to ensure that the medicinal preparation contains ingredients of the specified quality and quantity and follows standard analytical procedures to support the expected therapeutic effect [4]. Determination of the levels of efficacious substances in drug preparations is an essential part of agencies that carry out drug content determinations, such as the Food and Drug Supervisory Agency (BPOM) and industrial drugs, so fast and reliable analytical methods are needed as well as relatively inexpensive and accessible tools and operational costs.

In practice, but can provide results with good accuracy and precision. Spectrophotometry is a straightforward, quick, and relatively affordable method compared to other methods [5]. On the other hand, performing simultaneous quantitative analysis on pharmaceutical preparations that contain more than one active substance using the traditional spectrophotometric method can be challenging due to the overlapping spectra [6]. Widespread use of pharmaceutical preparations containing microelements requires weighty metal elements and a fast and precise ion tagging method. Spectrofluorometry, atomic absorption spectrophotometry (AAS), capillary electrophoresis, electrochemistry, thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), and ion chromatography were used to identify zinc metal as the most prevalent element in multi-mineral and multi-vitamin preparations with micro-elements found in biological materials, food, and pharmaceutical preparations. In particular, zinc metal was found to be [7].

HPLC, injection flow, voltammetry, NMR spectroscopy, fluorometric, and enzymatic methods are some of the analytical techniques used to determine the amount of vitamin C present in different samples.

The amount of vitamin C in a sample can be determined using various spectrophotometric techniques, including osazon, silver-gelatin complex, oxidation with iron (III), and reaction with toluidine blue and potassium iodate, among others [8]. Research conducted by Kustiawan and Pratiwi (2016) has analyzed Zn metal with Dhitizon 0.01% (w/v) by forming complex compounds that can be detected by UV.Vis spectrophotometry with a wavelength of 525 nm with the standard addition method [9]. Research conducted by Zareba S and Pomykalski A (2003) has determined Zn levels in the pharmaceutical preparation of Zincupin forte by UV.Vis spectrophotometry with a wavelength of 480 nm (pH 7.35) and 530 nm (pH 9.23) using methanol as a solvent: water (1:1). The choice of solvent is one thing that needs to be considered in the analysis using UV-Vis spectrophotometry [7]. This is due to some solvents being able to absorb UV-Vis radiation so that it can interfere with the results of the spectrum and the determination of the wavelength [10].Based on the description above, this research will perform solvent optimization and simultaneous analysis of Vitamin C and Zinc levels without any separation stages on tablet preparations by Visible Spectrophotometry.

II. METHODS

This research is experimental; that is, it is a research procedure that is carried out to reveal a causal relationship between two or more variables by controlling the influence of other variables; the independent variable induces the research object to find out the consequences of the dependent variable; and the dependent variable is the subject of the research [11].

Tools

UV-Vis 1800 spectrophotometer (Shimadzu) and a set of Personal Computer (PC) equipped with UV-Probe 2.42 software, Microsoft Excel and SPSS 20, Matlab® version R2016a, 1 cm cuvette, glass tools (Oberoi), mortar and pestle, rubber balls, analytical balance (Boeco), sonicator (Branson 1510), pH meter (Hanna) and other tools needed in preparing samples and solutions.

Materials

All reagents used are grade analysis unless otherwise stated. Raw Materials, Vitamin C (BPOM), Zinc (BPOM), water for injection (PT. Ika Pharmindo), Methanol (E-Merck), NaOH (E-Merck), Whatman filter paper no. 42, parchment paper, tablet S, tablet P.

Preparation of Test Solution

Preparation of 0.1 N Sodium Hydroxide Solvent

Dissolve as much as 4 g of NaOH in CO2-free distilled water, then add up to 1000 ml [12].

Preparation of 0.1 M Potassium Dihydrogen Phosphate Solution

Dissolved 6.805 g of potassium dihydrogen phosphate in water, made up to 500 mL with water for injection [13].

Preparation of a Phosphate Buffer Solution pH 4

Inject a total volume of 100 mL solution by combining 50.0 mL of 0.1 M potassium dihydrogen phosphate and 3.6 mL of 0.1 N sodium hydroxide. The solution should then be diluted with water [13].

Preparation of a Phosphate Buffer Solution pH 5

Inject a total volume of 100 mL solution by combining 50.0 mL of 0.1 M potassium dihydrogen phosphate and 4.6 mL of 0.1 N sodium hydroxide [13].

Preparation of a Phosphate Buffer Solution pH 6

Inject a total volume of 100 mL diluted with water and mixed with 50.0 mL of 0.1 M potassium dihydrogen phosphate and 5.6 mL of 0.1 N sodium hydroxide [13].

Solvent optimization

Optimization was carried out by measuring the absorption produced by Vitamin C and Zinc in methanol: water and a mixture of methanol: phosphate buffer pH 4; pH 5, pH 6.

Preparation of Zinc Standard Solution

After carefully weighing 50 mg of standard zinc, transfer it to a volumetric flask containing 50 ml. Standard Mother Solution I (LIB I) solution was obtained by dissolving the substance in a methanol and water mixture until it was completely dissolved, then adding the same solvent until it reached the line marked on the container. This was solved with a concentration of 1000 g/ml. Standard Mains Solution II is prepared by adding the same solvent to 5 milliliters of the LIB I solution that has been pipetted into a volumetric flask with a capacity of 50 milliliters. This will yield a zinc concentration of 100 micrograms per milliliter (LIB II).

Preparation of Vitamin C Standard Mains Solution

After carefully weighing 50 mg of the vitamin C standard, transfer it to a volumetric flask that has a capacity of 50 ml. They were dissolved in a methanol and water mixture until they were completely dissolved. Then a sufficient volume with the same solvent was added up to the marked line to get a solution with a concentration of 1000 g/ml. This solution is Standard Mains Solution I. (LIB I). In order to obtain a concentration of 100 g/ml Vitamin C solution, which is referred to as Standard Mains Solution II, pipette 5 ml of the LIB I solution into a volumetric flask that has a capacity of 50 ml, and then add the same solvent (LIB II).

Preparation of Maximum Zinc Absorption Spectrum

Pipette 2 ml of Zinc 100 µg/ml II standard mother liquor, put it into a 25 ml volumetric flask and add 2 ml of dithizone solution, add methanol and water to the marked line so that the concentration becomes eight µg/ml, then measure the maximum wavelength absorption in the 400-800 nm range.

Preparation of the maximum Absorption Spectrum of Vitamin C

Pipette 2 ml of LIB II Vitamin C, put it into a 25 ml volumetric flask and add methanol to the marked line. Shake until homogeneous to obtain a solution of Vitamin C with a concentration of 8 µg/ml.

Method Validation

Linearity, Limit of Detection (LOD) and Limit of Quantification (LOQ)

Pipette 1 mL, 1.5 mL, 2 mL, 2.5 mL, 2.75 mL, and 2.75 mL of LIB II Zinc into a 25 mL volumetric flask to get a concentration of 4, 6, 8, 10, and 11 g/mL. Then, using the solvent, fill the flask to the line that is marked on it. Each 1; 1.35; 1.8; 2.25; and 2.75 mL of LIB II Vit C should be pipetted into a 25 mL volumetric flask. Then, use a solvent to bring the volume up to the marked line to make a solution with a vitamin C concentration of 4, 5, 4, 7, 2, 9, or 11 g/mL. In the analysis of each substance, the level of absorption of each of the above solutions was looked at. Then, the relationship between the amount of each substance and how quickly it was absorbed was studied to get a linear regression equation and the correlation value.

y = a + bx

Based on the absorbance at λ analysis, LOD and LOQ were also calculated.

$$SD = \frac{\sqrt{\Sigma(Y - Yi)^2}}{n - 2}$$

$$LOD = \frac{3{,}3\ xSD}{Slope}$$

$$LOQ = \frac{10xSD}{Slope}$$

Information:

SD = Standard Deviation (Residual Standard Deviation)

Slope = b

2.9.2 Recovery

The test of recovery consisted of determining how much progress had been made by calculating the percentage of recovery in three distinct areas, namely 80%, 100%, and 120%. Whereas, in every particular area, 70% of the Vitamin C and Zinc samples were used from the analysed tablets, while the remaining 30% came from the raw material to be added. After that, the same method used to analyze the individual samples

(tablets) was applied to the mixture of samples and the standard. The recovery percentage is calculated using the formula:

$$Y = \frac{CF - CA}{CA*} x\ 100\%$$

Information:
Y = Recover percentage
CF = Measured Amount of analyte
CA = amount of analyte in the sample (70% comes from the sample)
CA∗ = amount of raw material added (30% comes from raw)

2.9.3 Precision testing

The SD or RSD of a data set expresses precision. To find RSD use the formula:

$$RSD = \frac{SD}{X} x\ 100\%$$

Information:
RSD = Relative standard deviation
SD = Standard deviation
X = Data that has been averaged

III. RESULT AND DISCUSSION

3.1 Solvent Optimization

Vitamin C and Zinc are soluble in a mixture of methanol: water with a ratio of 50:50 with a single test with a complexing agent, addition with a concentration of 0.1% producing a pink colour. While the comparator used was a mixture of methanol: phosphate buffer PH 4, 5, and 6 with the addition of a complexing agent dhitizon with a concentration of 0.1% producing a pink colour, but visually the solubility was not good, there were still many refined grains that did not dissolve evenly even though it has been sonified.

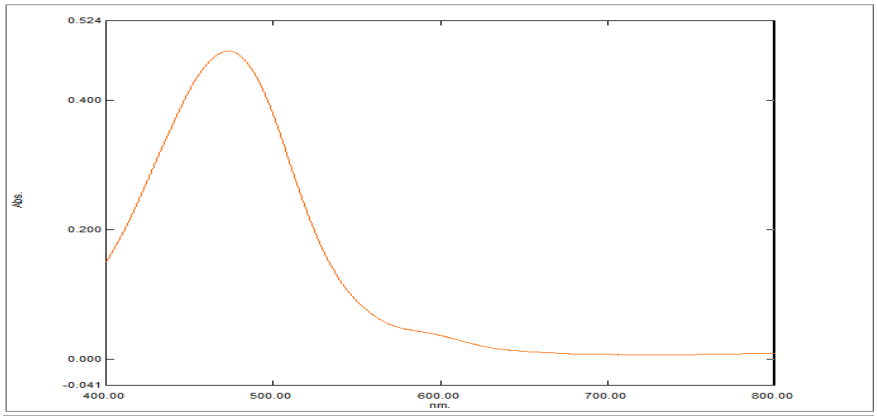

Fig 1. The maximum absorption spectrum of Vitamin C at λ 477 nm

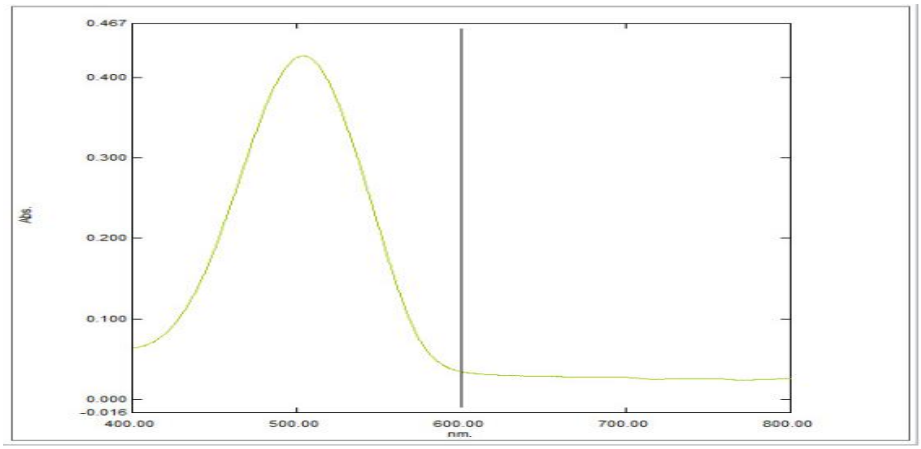

Fig 2. The maximum absorption spectrum of zinc

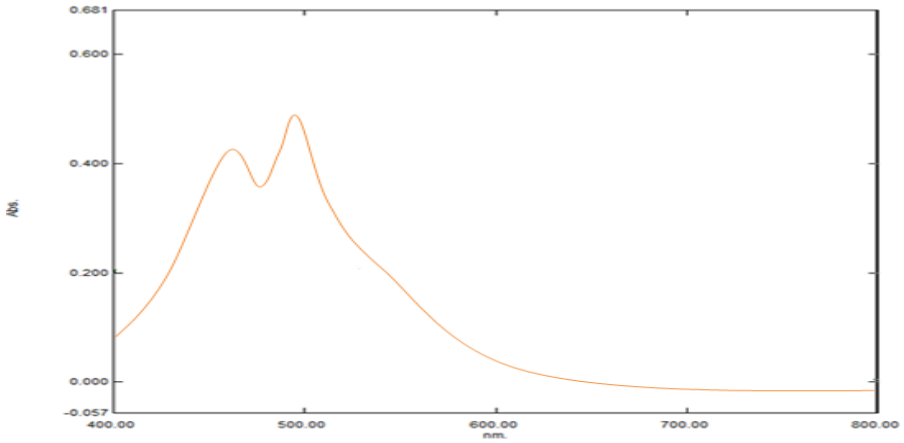

Fig 3. Mixed Absorption of Vitamin C and Zinc

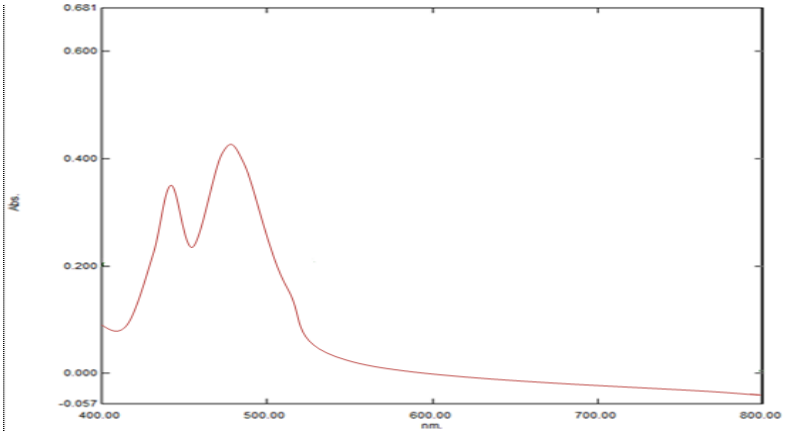

Fig 4. The spectrum of Tablet P

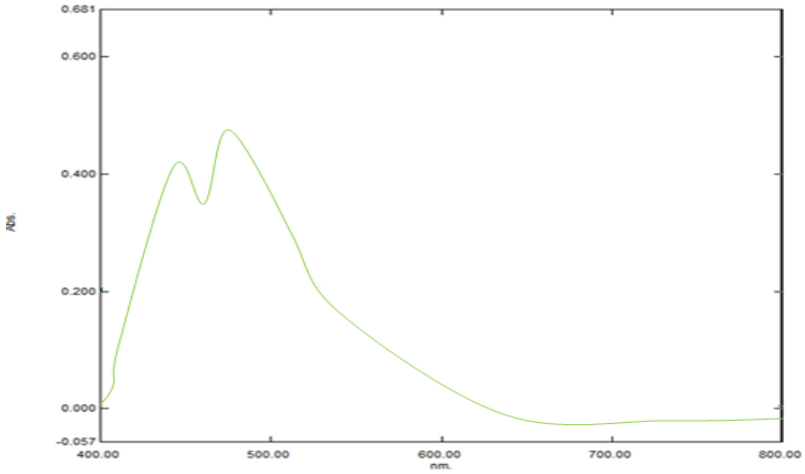

Fig 5. The spectrum of Tablet S

3.2 Linearity, accuracy, precision, the limit of detection (LOD) and limit of quantification (LOQ)

Based on the method validation carried out, the linearity, accuracy, precision, LOD and LOQ Visible Spectrophotometry values for Vitamin C and Zinc can be seen in Table 1.

Table 1. Value of linearity, accuracy, precision, LOD and LOQ for Vitamin C and Zinc

Parameter	**Vitamin C**	**Zinc**	**(FI Edisi V) (Christian, 1994)**
Linearity	0.9995	0,9998	≈ 1
Accuracy (%)	102.50	104.31	90-110%
Precision (RSD) (%)	1.44	3.58	< 5%
LOD (µg/mL)	0.42	1.00	-
LOQ (µg/mL)	1.29	3.05	-
Recovery	99.05%	104.31%	90-110%

The results of the validation of the analytical method are presented in Table 1. These results show that the method meets the requirements of linearity, accuracy, precision, the limit of detection (LOD), and quantification (LOQ). There is an excellent relationship or correlation between the drug concentration and the absorbance value, which is indicated by the linearity value, which is the value of the correlation coefficient for both Vitamin C and Zinc. This value is almost close to number one, which indicates that the linearity value is almost close to number one. Additionally, this suggests that the absorbance value will increase as the concentration rises.The values for the validation parameters are determined by the outcomes of the calculations, as presented in Table 1. The equation for linear regression, Y = 0.0607X + 0.0065, can be used to describe the calibration curve. The fact that the correlation coefficient (r) between concentration (X) and absorbance (Y) is 0.9995, as stated by the value, indicates that the correlation is strong. One parameter tested by adding a standard to a specific range in the sample is accuracy. This test is carried out to determine how accurate the parameter is. Both are measured, and then the standard that was added is recalculated. Sometimes a recovery test is performed instead of this test.

The value representing accuracy in Table 1 is the average return value obtained from three distinct ranges after three separate attempts.The precision method validation is a parameter that shows how close together the drug analysis results are when repeated multiple times. The precision demonstrates that the method produces comparable results regardless of the times it is put through its paces. In this particular instance, the three specific ranges that are being utilized are 80%, 100%, and 120%, and the composition is made up of 70% sample and 30% standard. According to the obtained accuracy value, this method successfully satisfies the requirements for method validation (the requirements for accuracy value are between 98% and 102%). The precision parameters are reflected in the RSD calculation values; based on the results obtained, both vitamin C and zinc satisfy the validation requirements (RSD of less than 5%) [14].The regression equation obtained from the calibration curve was used to calculate the limits of detection and quantification. The lowest concentration of analyte in the sample that can still be detected after reaching the limit of detection is referred to as the limit of detection. The lowest analyte concentration in a sample that still satisfies the criteria of being careful and thorough is the limit of quantification [15, 16]. This is the definition of the limit of quantification. The results of the detection limit are shown in Table 4.4. The detection limit for zinc is 1.00 µg/ml, and the detection limit for vitamin C is 0.42 µg/ml.

IV. CONCLUSION

The calibration curve for the spectrophotometric approach utilizing Dhitizon 0.1% + Mixture of Methanol: Water for standard Vitamin C and Zinc solutions The linear nature of visible spectrophotometry. By using visible spectrophotometry, the detection limits for vitamin C and zinc analysis were 0.42 mcg/ml and 1.00 mcg/ml, respectively. By using visible spectrophotometry, the quantitation limits for analyzing vitamin C and Zinc are 1.29 mcg/ml and 3.05 mcg/ml, respectively. With Dhitizon 0.1% + a mixture of methanol and water, the visible spectrophotometry method for analyzing vitamin C and Zinc has good precision and accuracy (valid).

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