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SPECTROPHOTOMETRIC DETERMINATION OF VITAMIN RIBOFLAVIN (Vit.B2) USING METHYLENE BLUE AS A COUPLING REAGENT

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Abstract: A low-cost method called spectrophotometry was developed to precisely determine vitamin riboflavin (Vit.B2) in both tablet and pure form. The result of the technique was a stable, soluble water-green product with absorption at 444 nm. It was predicated on an oxidative coupling reaction with potassium iodate and sulfuric acid present, utilizing methylene blue reagent. The following are the analytical data that were gathered during this: Beer's law holds at riboflavin concentrations between 0.5-27 µg/ml; molar absorbance is 8882.096 L/mol.cm, and Sandell's sensitivity is 0.042 µg/cm2. The standard deviation is relative and ranges from 0.151 to 0.714. Procedures for direct addition were applied to pharmaceutical standards and specimens, and the outcomes show that the recommended approach was effectively used to determine vitamin B2.

Keywords: determination; coupling reagent; methylene blue; riboflavin.

1. Introduction.

VitB2 (Riboflavin) produced for the first time in 1935 as a yellow-orange crystalline powder soluble in water [1]. One of the essential vitamins for bodily growth, riboflavin is a nutrient that may be obtained in food and as a supplement. It produces red blood cells, helps release energy from carbohydrates, contributes to cellular respiration, maintains the integrity of the mucous membranes of the skin and eyes, and produces antibodies, as well as average growth and development [2] . IUPAC name.

Dimethyl-10-[(2S,3S,4R)-2,3,4,5-tetrahydroxypentyl]benzo[g]pteridine-2,4-dione [3].-8‹7 . Its molecular weight and formula: $C_{17}H_{20}N_4O_6$, 376.369 g mol−1Structural formula of vitamin riboflavin[4].

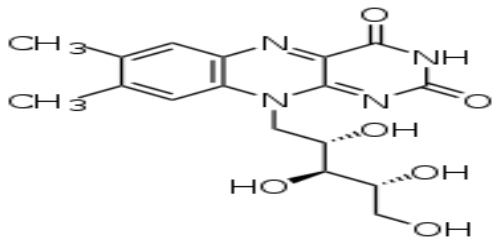


Fig. 1: Riboflavin VitB2's structural formula

It is essential for energy production, enzyme functions, and the formation of amino and fatty acids, and it scavenges free radicals [5]. Retrospective studies in children have suggested Teenagers who suffer from migraines also have some benefits associated with supplemental riboflavin [6-8]. Injections or oral nutritional supplements can avoid riboflavin shortage, which is uncommon and typically accompanied by deficits in vitamins and other minerals[9]. Since riboflavin is a water-soluble vitamin, the body doesn't store

it but uses it as needed. The rest is either rapidly eliminated in the urine after absorption or is not absorbed at all [10]. Riboflavin is found naturally in meat, fish, poultry, eggs, dairy products, green vegetables, mushrooms, and almonds. Certain nations demand the inclusion of specific grains [11]. Given the medical importance of this vitamin, it has been estimated using various analytical methods, such as Eco-Friendly quantification of riboflavin in biological and food [1], Spectrofluorimeteric [2], UV [5], LC-MS/MS [6, 7], UPLC-ESL-MS [9], HPLC [10, 11], electrochemical detection [8].

2. Experimental Part

All weight measurements were taken using a Sartorius Balance 210S kern and a spectrophotometer (T80+ Spectrometer UV/VIS+ instruments) fitted with 1 cm quartz cells.

2.1. Materials and solutions:

The materials used in this study were prepared in pure form from (US, FLUKA, Avonchem UK), and distilled water was used as the solution solvent.

1 - Standard solution (VitB2), 1000 µg/mL

In a volumetric flask, the volume was diluted to 100 ml using distilled water after dissolving 0.1000 g of vitB2, which has a molecular weight of 376.36 g.mol-1, in a small amount of distilled water. And be ready with another 200 µg/mL solution.

2- Methylene blue(MB(reagent solution, 1000 µg/mL .

A small amount of distilled water was used to dissolve 0.05 g of methylene blue powder (319.85 g.mol-1) in a 50 ml volumetric container. The container was then filled to the brim with distilled water. Distilled water was used to dilute 1 milliliter of this solution to 100 milliliters, yielding a ten µg/ml solution.

3- Potassium iodate solution (0.01)M.

A tiny amount of distilled water was used to dissolve 0.214 g of potassium iodate powder, which has a molecular weight of 214.001 g/ml. The volume was then filled to the mark with distilled water in a 100 ml volumetric container.

4 - Sulfuric acid solution (0.1M).

Sulfuric acid (0.2789)ml was diluted with distilled water in a 50-ml volumetric container to create this solution.

5- B-Plexin pharmaceutical solution.

Ten capsules of the pharmaceutical preparation B-Plexin, produced by BRAWN, were weighed, and they contained 5 mg of VitB1 and 2 mg of VitB2mg. The capsules were mixed well for homogenization, and their weight was 3.5511 grams; and they were dissolved in a volumetric vial Capacity of 100 ml with distilled water, then the solution was filtered using Whatman No.42 filter paper, the filtrate completed to the mark with the distilled water containing 200 µg/ml of riboflavin (vit B2)

3. Results and discussion:

3.1. Preliminary study

A 20 ml volumetric vial containing 0.5 ml of VitB2 200 µg /ml, 1 ml of the reagent (methylene blue) 10 µg /ml, 1 ml of hydrochloric acid 0.1 M, and 1 ml of potassium iodate at 0.01 M. After adding distilled water to fill the volume, a green solution Obtained with absorption at 444 nm, but the blank solution had no discernible absorption at the same wavelength.

3.2. Selected the optimum conditions

3.2.1. Effect of the oxidizing agent

1 ml of various oxidizing agents (0.01M), such as potassium iodate, iron(III) chloride, potassium iodide, and potassium chromate to 1 ml of methylene blue (10 µg/ml), to 1 ml of hydrochloric acid (0.1M), and 0.5 ml of VitB2 (200 µg. /ml). The results showed that the potassium iodate solution gave the highest absorption of the colored product at 444 nm, which was chosen in Table 1 in subsequent experiments.

Table 1: Effect of oxidizing agents

Oxidizing agent M $^{2-}$10×1	Absorption
Iron(III) chloride	0.219
Potassium iodide	0.224
Potassium iodate	0.225

3. 2.2. Effect of the volume of oxidizing agent

By adding varying volumes (0.25 - 2 ml) of the oxidizing agent in a 20 ml volumetric vial containing 1 ml of reagent (methylene blue ten µg/ml), 1 ml of hydrochloric acid (0.1M), and 0.5 ml of VitB2 (200 µg. /ml). The optimal amount of the oxidizing agent, potassium iodate (0.01), was tested. Using pure water, the volume was lowered to 20 ml. Because of its increased absorption, the volume of 1 ml of the oxidizing agent (0.01) was found to be the ideal quantity in Table (2).

Table 2: Effect of the amount of oxidizing agent

KIO_3 1×$^{2-}$10^{-}M volume ml	Absorption
0.25	0.215
0.50	0.220
1	**0.225**
1.5	0.221
2	0.201

3.2.3. The effeCt of acid.

Solutions Of acids (HCl, H_2SO_4, HNO_3, CH_3COOH) were prepared Conc. of 0.1 M. Select the type of medium needed to complete the reaction process.

Table 3: The effect of acid.

Acid type	Absorption
CH_3COOH	0.222
H_2SO_4	0.229
HCl	0.225
HNO_3	0225.

It is noted from The table above that sulfuric acid gave the highest absorbance of the colored product at 444 nm, so it was used as the best acid.

3.2.4. The effect of the amount of acid.

By adding varying quantities (0.25-2) ml of acid to a volumetric vial Containing 1 ml of reagent (10 µg/ml), 1 ml of oxidizing (0.1), and 0.5 of vit B2 (200 µg/ml Distilled water was added to the capacity to make it 20 milliliters, the data in Table (4) suggested that 1 ml of acid was the ideal volume, and this was selected.

Table 4: The effect of the amount of acid.

H_2SO_4 1×10^{-1}M	Absorption
0.25	0.211
0.5	0.227
1	0.229
1.5	0.218
2	0.208

3.2.5. Effect of The amount of MB reagent.

A 20 ml volumetric vial was filled with varying quantities of reagent MB (0.25-2 ml) of the reagent solution (10 µg/ml), followed by 1 ml of acid (0.1M) and 0.5 ml of VitB2 (200 µg. /ml). This allowed for the study of the influence of reagent quantity. Add distilled water to the mark of volume. The findings presented in Table (5) indicate that 1 ml is the ideal volume of reagent (MB) (10 µg/ml).

Table 5 Effect of reagent quantity.

Reagent (10 µg/ml)	Absorption
0.25	0.225
0.5	0.226
1	0.23
1.5	0.228
2	0.225

3.2.6. Effect of temperature

The effect of temperature(5- 550°C) on the absorption of the formed colored product is studied. One ml of reagent MB at a conc of 10 (µg/ml) was used, along with one ml of oxidizing agent and one ml of acid (0.1), and finally, 0.5 ml of VitB2 (200 µg/ml). After that, it was put into several 20 ml volumetric bottles, and added distilled water. The optimum temperature was 25C°, which was the temperature in the laboratory.

Table 6: Study of the effect of temperature.

Temperature	Absorbency
5	0.11
10	0.13
15	0.16
18	0.23
25	0.229
30	0.228
35	0.225
40	0.117
50	0.114
55	0.108

3.2.7. The effect of the addition sequence

The impact of adding the solutions correctly to the final color's intensity was investigated. Since the same ideal circumstances were used in the earlier studies, as indicated in Table 7, several tests were carried out using various addition sequences utilizing 20 ml volumetric bottles.

Table 7: Effect of addition sequence.

Absorption	Order of addition	NO
0.19	M+B+E+H	1
0.18	B+H+E+M	2
0.20	H+B+M+E	3
0.23	M+H+E+B	4

M=Methylene Blue،E=KIO_3،H=H_2SO_4،B=B2

3.2.8. Effect of TiMe on Stability of the Colored Product.

Utilizing a volume of 1 ml of reagent MB at a concentration of 10 µg/ml, 1 ml of acid (0.1M), 1 ml of oxidizing agent (0.01), and 0.5 ml of VitB2 200 (µg/ml), the impact of stability time on the absorption of the finished product was examined. The mixture was put into several volumetric 20 ml bottles, and the capacity was completed with distilled water. The duration of the trial was 0–60 minutes. It was found that the solution remained stable for at least half an hour at a laboratory temperature of no more than 25 C0.

Table 8: Stability time.

Time	Absorption
0	0.229
10	0.229
15	0.229
18	0.229
25	0.229
30	0.229
35	0.228
40	0.229
50	0.228
60	0.228

3.3. Final absorption spectrum

The final absorption spectrum was measured after reaching the optimal conditions, which are using 1 ml of methylene blue reagent solution, 1 ml of sulfuric acid solution, 1 ml of potassium iodate oxidizing agent solution, and 0.5 ml of VitB2 solution, and filling a 20-volume vial up to the full mark. After adding distilled water to fill, compare the green color's absorbance to its blank solution, which has the maximum absorption at 444 nm. There was no absorption in this area from its blank Solution. figure 2.

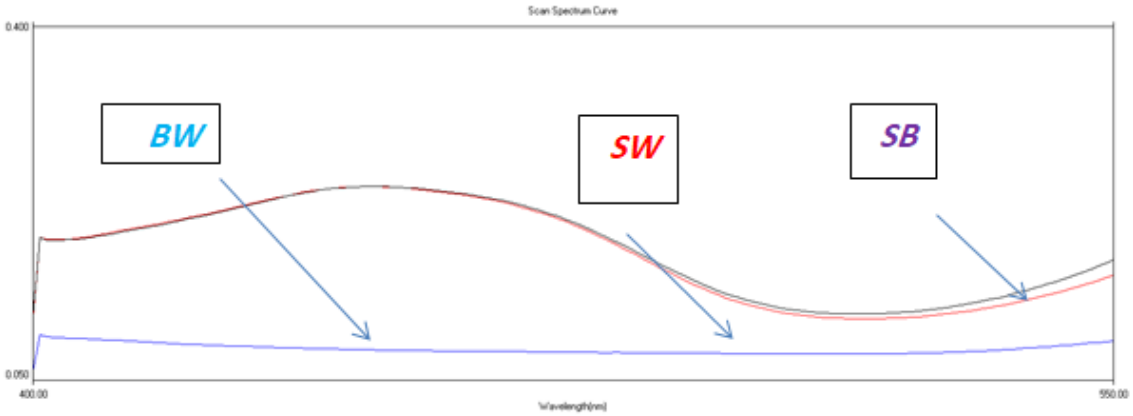


Fig. 2:

SW - the B2 model solution's absorption spectra compared to water.

SB - the model solution B2's absorption spectra compared to the blank solution.

BW - the blank solution's absorption spectrum compared to pure water.

3.4 Calibration Graph and the Statistical Data.

A calibration graph was constructed using the determined ideal circumstances (Fig.3). The graph indicates that the green product satisfies Beer's law when applied to a succession of 20 ml volumetric flasks containing 1 ml each of the oxidizing agents KIO3 0.01M and H2SO4 0. 1M at escalating concentrations (0.5–27 µg/ml).1 milliliter of 10 µg/ml methylene blue reagent was added, and distilled water was used to fill the container to the entire line. Following that, each solution's absorbance was measured at a wavelength of 444 nm, as the calibration curve for VitB2 is displayed in Figure (3).

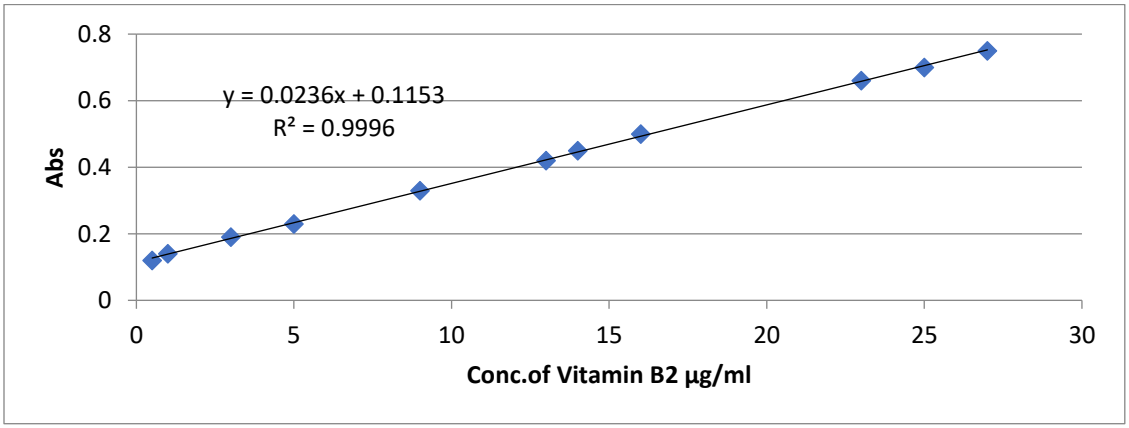


Fig. 3: Calibration curve for determination of B2) by oxidative coupling with MB reagent.

3.4. Accuracy and precision

The calibration curve was designed to verify the method's accuracy and precision under optimum conditions. Within Beer's Law, three VitB2 concentrations were measured for the calibration curve. The relative standard deviation was (0.714, 0.168, 0.151%), and the average Rec.% was (101.21), indicating high precision and accuracy in the procedure—table (9).

Table 9: Accuracy and precision.

RSD %	Average of Recovery %	Recovery %	Conc. of B2 µg/ml(observed)	Conc. Of Vit. B2 µg/mL
0.714	101.21	104	1.04	1
0.168		99.30	12.91	13
0.151		100.34	23.08	23

3.5. Calculate the qualitative and quantitative detection limit

The detection limit for determining VitB2 at the wavelength of 444nm was calculated by measuring the Blank solution against the water ten Times, where the qualitative and quantitative detection limits were as in Table (10).

Table 10: Detection limit

SIope	SD	LOD µg/ml	LOQ µg/ml.
0.0236	0.000316	.04410	0.133

3.6. Stoichiometry of Reaction

The molar ratio method and the continuous changes method (JOB) were used to ascertain the type of the final product and the rate at which the VitB2 component was bound to the reagent. MB solution has the same concentration in both, which is $1x^{-4}10$ M.

The results appeared as in Figure (4 , 5), showing that the ratio is (1:1).

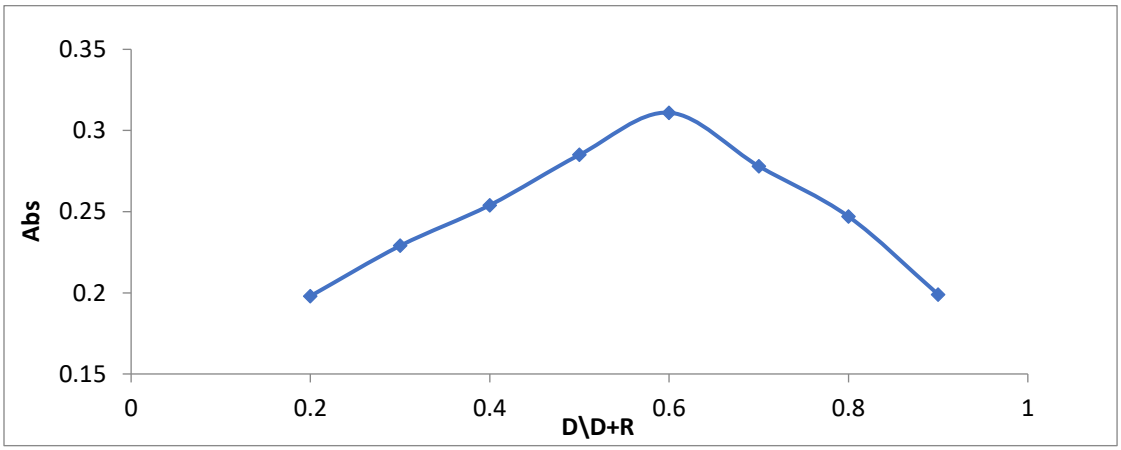


Fig. 4: Job method

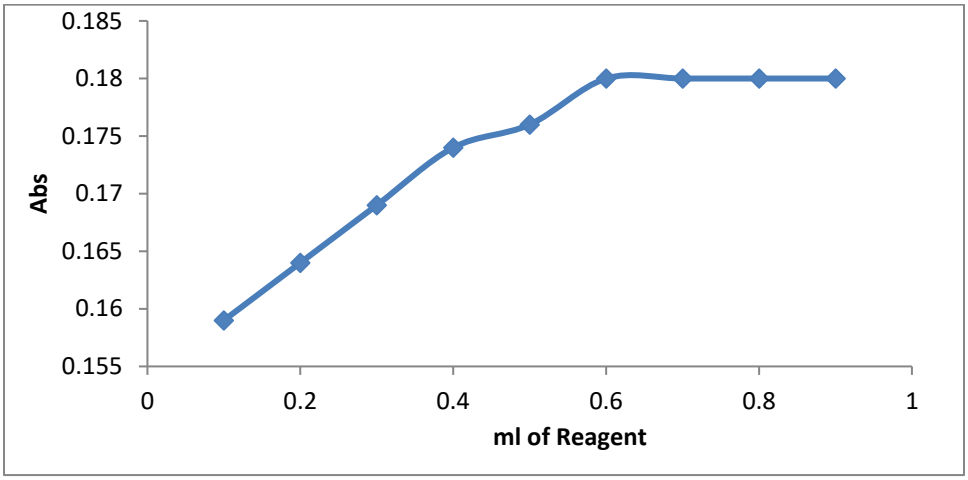


Fig. 5: Molar ratio method.

4. Application

1.4. Direct method:

Three concentrations of the pharmaceutical formulation solution (200 µg/ml), 3, 5, and 9 µg/ml were used in this approach. After transferring the solutions to a 20 ml vial and treating them according to the protocols outlined in the calibration curve, the absorbance at 444 nm was determined. The effectiveness and success of the procedure in its pharmaceutical formulation. Table (11)

Table 11: Direct method for determining VitB2

Conc. of VitB2 µg\ml	Conc. of VitB2 µg\ml Found	Recovery %	Re %
3	2.9	96.6	3.3
5	4.86	97.2	2.8

Determining ion:

A simple, sensitive, fast spectroscopic method has been developed to estimate VitB2 in its pure form and pharmaceutical preparations. The process is based on oxidative coupling between the reagent methylene blue and VitB2 in the presence of sulfuric acid and potassium iodate to form a green solution to obtain a solution dissolved in water and stable and showing maximum absorption at 444 nm. The current method is simple because it does not require heating, hydrolysis, or extraction using organic solvents. This makes it a successful method for VitB2 in its pure form and pharmaceutical preparations.

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