IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 3, 2022

Analytical Estimation of Riboflavin Using UV Spectroscopy with Various Buffer Solutions

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Abstract

The detection and assay of vitamin B-2 (Riboflavin) were conducted under aqueous conditions using various buffer solutions such as citrate buffer and sodium borate buffer at different pH levels. The absorbance spectrum of Riboflavin was measured across different pH values using these buffers. A spectrophotometer was utilized to achieve accurate and sensitive assay of Riboflavin at a wavelength of 440 nm. For preparation, a stock solution of Riboflavin with a concentration of 1.403 × 10−4 molar was prepared by dissolving the appropriate amount of the vitamin. Measurements included various aqueous solutions containing Riboflavin, such as aqueous test samples, vitamin capsules/tablets, and vitamin-water mixtures. UV/VIS spectrophotometry at 440 nm using different buffer solutions proved effective for assaying the B vitamin Riboflavin in aqueous media.

Key Words: Riboflavin, Buffer solutions, UV/VIS spectrophotometry.

INTRODUCTION

The term "vitamin" originates from the Latin word "vita," meaning life. Riboflavin (vitamin B2) is a water-soluble vitamin that fluoresces yellow-green and imparts a yellow color to B vitamin supplements. Vitamins typically function as catalysts, co-enzymes, or integral components of coenzymes. Dietary sources of vitamins include dairy products, fish, rice, wheat, egg yolk, vegetables, fortified cereals, chicken, and various meats. Ascorbic acid protects Riboflavin from degradation. Deficiency in each vitamin can lead to different associated diseases.

Vitamins are categorized into water-soluble and fat-soluble types based on their solubility and chemical properties. Analytical methods such as UV-VIS spectrophotometry and spectrofluorimetry have been optimized for the simultaneous quantification of vitamins.

However, these methods often involve chemical reactions or separation steps, which can introduce human error and increase the cost of analysis. Riboflavin (vitamin B2), for instance, is considered non-toxic when consumed because of its lower aqueous solubility [1], and any excess amounts are readily excreted in the urine.

Riboflavin is commonly used as a supplement in tablet or capsule form and is frequently added to energy drinks and similar health-related beverages. Its application in clinical and therapeutic settings has been explored in limited studies. For instance, higher doses of Riboflavin have shown promise in preventing migraine headaches. When combined with UV light in blood products, Riboflavin reduces pathogen activity by inhibiting replication [2]. Riboflavin has also been effective against nuclear cataracts. Various methods have been developed to determine Riboflavin content. These include isocratic reversed-phase column high-performance liquid chromatography (HPLC) with fluorometry detection, fluorescence detection following HPLC [3], and direct fluorescence assays after sample pretreatment. Planar chromatography has also been utilized for the simultaneous detection of multiple vitamins, including Riboflavin, followed by fluorescence and UV-visible detection, confirmed by electrospray ionization mass spectrometry.

MATERIAL AND METHODOLOGY

2.1 Materials:

2.1.1 Riboflavin Drug Standard:

Riboflavin Drug Standard was generously donated by Popular Pharmaceuticals Ltd., India.

2.1.2 Dosage Method:

Riboflavin tablets (10mg) were procured from a local drug store in Dharmapuri. The samples were carefully checked for manufacturing license numbers, batch numbers, production, and expiry dates. They were randomly coded as A, B, and C, and stored under appropriate conditions.

2.1.3 Reagents:

This study employed buffer solutions (citric acid buffer, sodium borate buffer, phosphate buffer) and distilled water.

2.1.4 Instruments:

The instruments used included a single pan balance and a UV/visible spectrophotometer.

2.2 Procedure:

2.2.1. By using citric acid buffer:

Preparation of standard and test solution:

Riboflavin solid consists of orange crystals and produces a yellow aqueous solution. A stock solution of Riboflavin were prepared by dissolving 0.0526 g into one liter of distilled water making a conc. of $1.403\times10-4$ molar. This container was wrapped in aluminum foil to protect Riboflavin stock solution from light exposure. In the case of Tablet or capsule:

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1) The tablet is weighed
2) Then ground in mortar and pestle
3) The dry amount of solid should be dissolved in volumetric flask.
4) The desired amount of solid is carefully placed in volumetric flask and dissolved in distilled water
5) Then filter in gout insoluble solid through Whatman #1filter paper
6) The filtered liquid is ready for assay

2.2.2. By using sodium borate buffer:

Preparation of standard and test Solution:

Riboflavin solid is composed of orange crystals, yielding a yellow aqueous solution. To prepare a stock solution, 0.0526 g of Riboflavin was dissolved in one liter of distilled water, resulting in a concentration of 1.403×10^{-4} molar. The container containing the stock solution was wrapped in aluminum foil to shield it from light exposure [4].

A sodium borate buffer solution with a concentration of 0.110 molar was prepared in distilled water, adjusted to a pH value of 7.54. This was intended to assess the feasibility of measuring vitamin content in industrial or beverage aqueous mixtures. For tablets or capsules:

- Weigh the tablet.
- Grind it using a mortar and pestle.
- Dissolve the measured amount of solid in a volumetric flask containing distilled water.
- Ensure all solid material is dissolved in the volumetric flask.
- Filter out any insoluble solids using Whatman #1 filter paper.
- The filtered liquid is now prepared for assay.

2.2.3. Using Phosphate Buffer:

Preparation of Standard and Test Solutions:

Riboflavin solid consists of orange crystals and produces a yellow aqueous solution. A stock solution of Riboflavin was prepared by dissolving 0.0528 g in one liter of distilled water, resulting in a concentration of 1.403×10^{-4} molar. The container containing the Riboflavin stock solution was wrapped in aluminum foil to protect it from light exposure [5].

A stock solution of Phosphate buffer with a concentration of 0.010 molar was prepared in distilled water at a pH value of 6.8.

For tablets or capsules:

- Weigh the tablets.
- Grind them using a mortar and pestle.
- Dissolve the measured amount of solid in a volumetric flask.
- Carefully place the desired amount of solid in a volumetric flask and dissolve it in distilled water.
- Filter out any insoluble solids using Whatman #1 filter paper.
- The filtered liquid is now prepared for assay.

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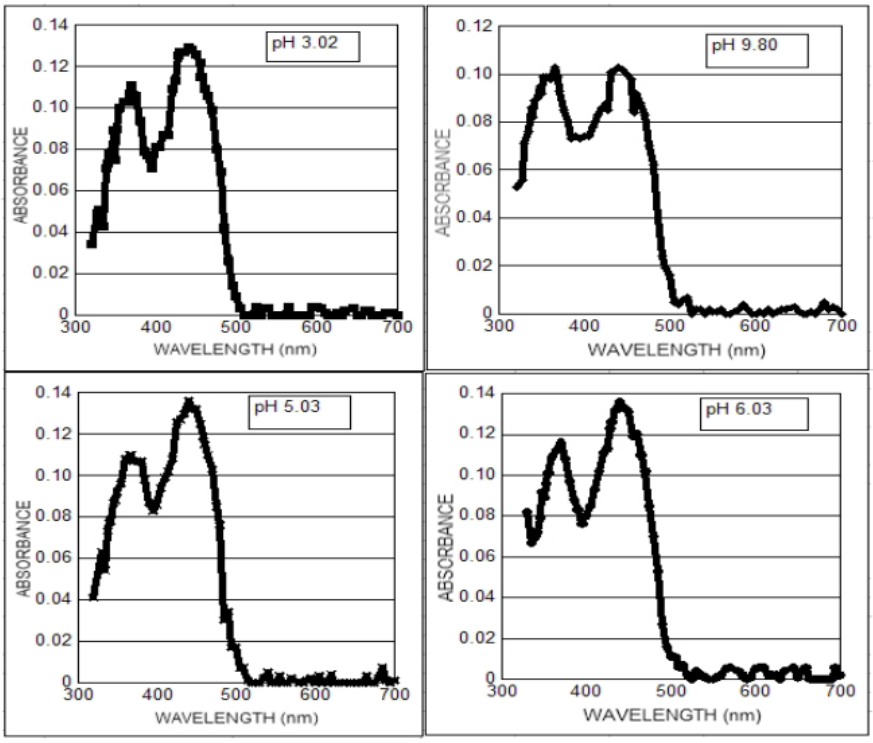
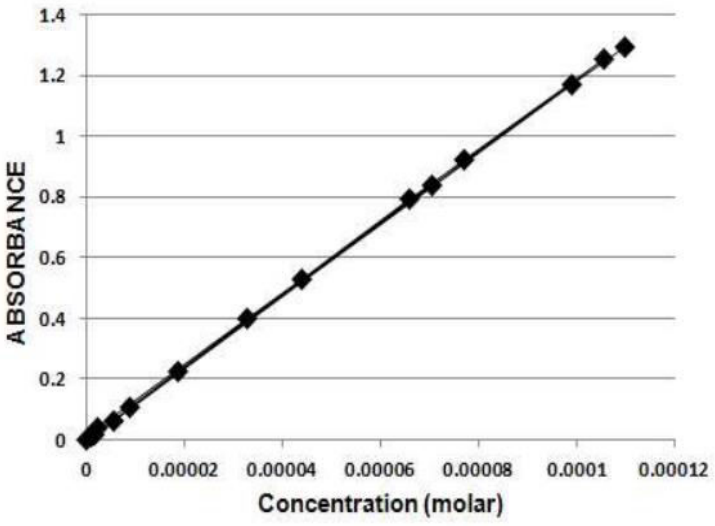
RESULTS

3.1 By citric acid buffer:

Riboflavin is a yellow to orange crystal-like powder with a slight odor. The solid itself is not significantly affected by light, but once in solution, the vitamin degrades quickly under light exposure. The average percent recovery of Riboflavin was 101.9%, with a standard deviation of 1.1%. The minimum percent recovery observed was 100.25%, and the maximum was 103.7%. The median percent recovery was 102.8%, and the values are approximately symmetric with a skewness of 0.152. The coefficient of variation for percent recovery is 1.1%.





The standard curve exhibits a linear equation of y = 11872x with a coefficient of determination (R^2) of 0.9988 and a Pearson correlation coefficient (r) of 0.9998.

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3.2 By using sodium borate buffer:

Riboflavin has a variable but low solubility in water approximately 1 mg in 20 ml The variability is attributed to differences in the internal crystalline structure of Riboflavin. The line equation was y = 12546 (including the intercept), with an essential correlation coefficient of 1.100 and a coefficient of determination $R^2 = 1.100$. The standard deviation of the slope is 1.66, and the 95% confidence interval for the slope ranges from 12533 to 12565.

3.3. Using Phosphate Buffer:

Riboflavin is a yellow to orange crystalline powder. The solid is not significantly affected by light, but when in solution, the vitamin degrades quickly under light exposure. The percent recovery of Riboflavin averaged 105.9%, with a standard deviation of 1.2%. The minimum percent recovery was 103.25% and the maximum was 108.7%. The median percent recovery was 107.8%, and the distribution is approximately symmetric with a skewness of 0.151. The coefficient of variation for percent recovery is 1.1%.

CONCLUSION:

Various aqueous mixtures containing Riboflavin were successfully measured, including aqueous test samples, vitamin capsules/tablets, and water vitamin mixtures. This method is rapid, widely applicable due to Riboflavin's aqueous solubility, easy to implement, suitable for quality control, and efficient for manufacturing processes. In conclusion, vitamin B-2 (Riboflavin) can be reliably assayed using UV/VIS spectrophotometry at wavelengths ranging from 310 nm to 440 nm in aqueous media with different buffer solutions at various pH values.

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