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Article

# Spectrophotometric Determination of Riboflavin Using Sodium Nitroprusside as Chromogenic Agent

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**ABSTRACT**

A simple, sensitive, and accurate spectrophotometric method has been developed for the determination of riboflavin in pure and pharmaceutical formulations. The method is based on the reaction of riboflavin with sodium nitroprusside in the presence of hydroxylamine hydrochloride and sodium carbonate to give an intense greenish yellow colored species which absorb maximally at 445 nm. Beer's law is obeyed in the concentration range of 0.94–7.52 ppm with a coefficient of determination $r^2$=0.9996. Statistical analysis of this method exhibited relative standard deviation of 1.28% with the limit of detection 0.564 ppm and limit of determination 1.690 ppm. The proposed method has been applied successfully for the determination of riboflavin in pharmaceutical products and can be extended for the routine analysis in bulk drugs.

**Key words:** Riboflavin, Spectroscopy, Hydroxylamine hydrochloride.

## 1. INTRODUCTION

Riboflavin is also known as Vitamin-B2 with molecular formula $C_{17}H_{20}N_4O_6$. The chemical name of riboflavin is [7,8-dimethyl-70-[(2s,3s,4r)-2,3,4,5-tetrahydroxy pentyl]benzo[g] pteridine-2,4-dione (Figure 1). It is an easily absorbed colored micronutrient with a key role in maintaining health in humans, animals, and in energy metabolism for the metabolism of fats, ketone bodies, carbohydrates, and proteins [1-5]. The name "riboflavin" comes from ribose, the sugar whose reduced form, ribitol forms part of its structure and "flavin," the ring moiety which imparts the yellow color to the oxidized molecule. It is the central component of the cofactors Flavin mononucleotide and flavin adenine dinucleotide. Reduction of isoalloxazine ring yields the reduced forms of the flavoproteins exhibit a wide range of redox potentials, play an important role in the electron transport chain, conversion of retinol to retinoic acid and tryptophan to niacin. The important sources of riboflavin are liver, kidneys, legumes, yeast, mushrooms, almonds [1], cheese, asparagus, yogurt, meat, fish, cereals, bananas, and wheat bran, riboflavin is used as an orange-red food color additive, designated in Europe as E101. It is used in baby foods, pastas, sauces, and fruit drinks. The clinical uses include high doses riboflavin along with beta-blockers are used in the prevention of migraine [2]. Riboflavin and ultraviolet (UV) treatment is effective for inactivating pathogens in platelets and plasma [3]. In humans, signs and symptoms of riboflavin deficiency include cracked and red lips, inflammation of the lining of mouth and tongue, mouth ulcers. It also leads to cause visual disturbances, cataract formation, burning, and itching of mucous membranes. Angular cheilitis, photophobia, and scrotal dermatitis are the classical remembered signs. The deficiency also causes continuous excretion in the urine of healthy individuals [4]. Other names of riboflavin were B-complex vitamin, lactoflavin, flavin, and Vitamin-G.

Spectrophotometry is one of the best and useful techniques for the determination trace level concentrations of the various metal complexes, metal ions, drugs, and chemicals [5-11]. Hence, in the present investigation spectrophotometry is used for the determination of riboflavin using sodium nitroprusside as chromogenic agent.

## 2. EXPERIMENTAL

### *2.1. Materials*

Riboflavin obtained from Fluka, India. Sodium nitroprusside, sodium carbonate, hydroxylamine hydrochloride, and dimethylformamide revived form S. D. Fine, India. Double distilled water is used throughout the experiments.

### *2.2. Standard and Sample Solution of Riboflavin*

0.3764 g of riboflavin were weighed into a 100 mL beaker. 20 mL of distilled dimethylformamide were added and stirred well. The resultant solution was gently heated on water bath to complete the dissolution. This was cooled and quantitatively transferred into a 100 mL volumetric flask. The solution was made up to the mark with distilled dimethylformamide to get 0.01 M solution. The working solutions were prepared by diluting the stock solution appropriately with dimethylformamide.

### *2.3. Sodium Nitroprusside*

A 0.01 M solution of sodium nitroprusside was prepared by dissolving 0.2619 g of sodium nitroprusside in distilled water and then diluted to the volume in a 100 mL volumetric flask.

### *2.4. Hydroxylamine Hydrochloride*

0.1 M solution of hydroxylamine hydrochloride was prepared by dissolving 0.6949 g of the salt in 100 mL of distilled water. Lower

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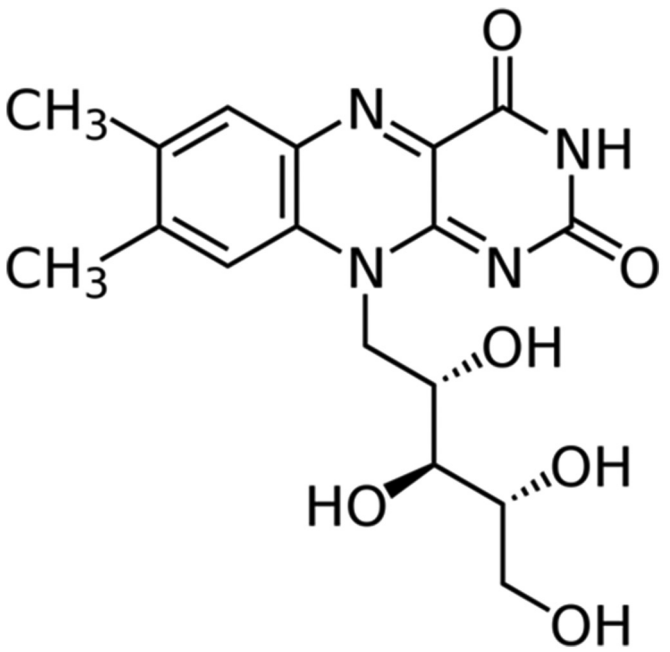


**Figure 1:** Structure of riboflavin.

concentration was prepared by diluting the stock solution appropriately with distilled water.

### *2.5. Sodium Carbonate*

1.0600 g of sodium carbonate were dissolved in distilled water and made up to the mark in a 100 mL volumetric flask which gives 0.1 M solution.

### *2.6. Determination of Riboflavin Using Sodium Nitroprusside, Hydroxyl Amine Hydrochloride and Sodium Carbonate*

Riboflavin reacts with sodium nitroprusside in the presence of hydroxylamine hydrochloride and sodium carbonate forming an intense greenish yellow colored solution. A spectrophotometric method was developed by analyzing the colored solution for the determination of riboflavin.

### *2.7. Absorption Spectra of Riboflavin – Sodium Nitroprusside Mixture and Reagent Blank*

0.5 mL of riboflavin ($1\times10^{-3}$ M), 1 mL of sodium nitroprusside ($1\times10^{-2}$ M), 1 mL of hydroxylamine hydrochloride ($1\times10^{-2}$ M), and 1 mL of sodium carbonate ($1\times10^{-2}$ M) were mixed in a 10 mL of volumetric flask. The contents are shaken well and made up to the mark with distilled water. In another 10 mL volumetric flask, 1 mL of sodium nitroprusside ($1\times10^{-2}$ M), 1 mL of hydroxylamine hydrochloride ($1\times10^{-2}$ M), and 1 mL of sodium carbonate ($1\times10^{-2}$ M) are mixed and diluted to the volume with distilled water. A greenish yellow colored solution was resulted. The absorbance of the resultant solution was measured in the wavelength region 300–500 nm against reagent blank for the experimental solution and against water blank for the reagent solution. The data are tabulated in Table 1. Absorption spectra were drawn for the reagent solution and the reaction mixture by plotting wavelength against the measured absorbance values. The plots reveal that the reaction mixture exhibits maximum absorbance at 445 nm where the reagent solution shows no absorption. Hence, all the further studies were carried at 445 nm against the reagent solution blank.

### *2.8. Effect of Reagent Concentration on Absorbance*

The effect of the reagent on the color intensity of experimental solution was studied using the following procedure.

**Table 1:** Absorption spectra of (a) riboflavin-reagent mixture and (b) reagent blank solutions {$[Fe(CN)_5NO)]^{2-}=1\times10^{-3}$ M; $[C_{17}H_{20}N_4O_6]=5\times10^{-4}$ M; $[NH_2OH.HCl]=1\times10^{-3}$ M; $[Na_2CO_3]=1\times10^{-3}$ M}.

| Wavelength (nm) | Absorbance (a) | Absorbance (b) |
|---|---|---|
| 300 | 0.245 | 0.022 |
| 305 | 0.215 | 0.020 |
| 310 | 0.211 | 0.015 |
| 315 | 0.219 | 0.005 |
| 320 | 0.257 | 0.005 |
| 325 | 0.304 | 0.002 |
| 330 | 0.361 | 0.004 |
| 335 | 0.423 | 0.015 |
| 340 | 0.490 | 0.008 |
| 345 | 0.545 | 0.004 |
| 350 | 0.594 | 0.005 |
| 355 | 0.630 | 0.002 |
| 360 | 0.673 | 0.003 |
| 365 | 0.699 | 0.002 |
| 370 | 0.705 | 0.006 |
| 375 | 0.707 | 0.010 |
| 380 | 0.677 | 0.007 |
| 385 | 0.622 | 0.004 |
| 390 | 0.561 | 0.008 |
| 395 | 0.513 | 0.003 |
| 400 | 0.496 | 0.002 |
| 405 | 0.509 | 0.002 |
| 410 | 0.546 | 0.002 |
| 415 | 0.593 | 0.002 |
| 420 | 0.642 | 0.002 |
| 425 | 0.690 | 0.002 |
| 430 | 0.737 | 0.003 |
| 435 | 0.782 | 0.002 |
| 440 | 0.819 | 0.001 |
| 445 | 0.838 | 0.000 |
| 450 | 0.833 | 0.002 |
| 455 | 0.812 | 0.003 |
| 460 | 0.777 | 0.002 |
| 465 | 0.738 | 0.003 |
| 470 | 0.688 | 0.001 |
| 475 | 0.620 | 0.002 |
| 480 | 0.533 | 0.002 |
| 485 | 0.431 | 0.001 |
| 490 | 0.328 | 0.001 |
| 495 | 0.235 | 0.001 |
| 500 | 0.161 | 0.001 |
| 505 | 0.108 | 0.001 |
| 510 | 0.073 | 0.001 |
| 515 | 0.051 | 0.001 |
| 520 | 0.039 | 0.001 |
| 525 | 0.032 | 0.001 |

0.5 mL of riboflavin ($1\times10^{-3}$ M) taken in each of a set of different 10 mL volumetric flasks, 1 mL of hydroxylamine hydrochloride ($1\times10^{-2}$ M), 1 mL of sodium carbonate ($1\times10^{-2}$ M), and different volumes of sodium nitroprusside ($1\times10^{-2}$ M) were added and made up to the mark with distilled water. The absorbance was measured at 445 nm against the corresponding reagent solution blank. The data are tabulated in Table 2.

The results in the above table indicate that the color intensity of the reaction mixture is not very much dependent on the variation in sodium nitroprusside concentration. However, a twenty fold excess of nitroprusside concentration was maintained in the further studies.

### *2.9. Applicability of Beer's Law*

The absorbance of the solutions containing various amounts of riboflavin (0.94–7.52 ppm), 0.4 mL of sodium nitroprusside ($1\times10^{-2}$ M), 1 mL of hydroxylamine hydrochloride ($1\times10^{-2}$ M), and 1 mL of sodium carbonate ($1\times10^{-2}$ M) in a total volume of 10 mL was measured at 445 nm against reagent blank. The absorbance data are tabulated in Table 3.

The plot drawn between the absorbance values and amount of riboflavin showed a straight line in the range of 0.94–7.52ppm of riboflavin. The absorbance data were subjected to statistical treatment. The linear plot with a slope 0.0217, y-intercept 0.011 gave a coefficient of determination $r^2$=0.9996. The detection and determination limits were obtained as 0.564 ppm and 1.690 ppm, respectively. The statistical data confirm the validity of the proposed method for the determination of riboflavin.

### *2.10. Instrumentation*

After due calibration of the instrument, spectral and absorbance measurements were made using UV-visible spectrophotometer model-UV-160A, Shimadzu Corporation spectrophotometric instrument plant, Kyote, Japan. All the solutions were freshly prepared for analysis.

**Table 2:** Effect of sodium nitroprusside concentration on absorbance of riboflavin {$[Fe(CN)_5NO]^{2-}=1\times10^{-2}$ M; $[C_{17}H_{20}N_4O_6]=5\times10^{-4}$ M; $[NH_2OH.HCl]=1\times10^{-3}$ M;$[Na_2CO_3]=1\times10^{-3}$ M}.

| Volume of sodium nitroprusside (mL) | Absorbance (445nm) |
|---|---|
| 0.25 | 0.692 |
| 0.50 | 0.699 |
| 0.75 | 0.710 |
| 1.00 | 0.718 |
| 1.25 | 0.725 |
| 1.50 | 0.732 |
| 1.75 | 0.737 |
| 2.00 | 0.745 |

**Table 3:** Beer's law data {$[Fe(CN)_5NO]^{2-}=4\times10^{-4}$ M; $[NH_2OH.HCl]=1\times10^{-3}$ M, $[Na_2CO_3]=1\times10^{-3}$ M}.

| Amount of riboflavin (ppm) | Absorbance (445nm) |
|---|---|
| 0.94 | 0.032 |
| 1.88 | 0.052 |
| 2.82 | 0.073 |
| 3.76 | 0.091 |
| 4.70 | 0.112 |
| 5.64 | 0.134 |
| 6.58 | 0.154 |
| 7.52 | 0.175 |

## 3. RESULTS AND DISCUSSION

Riboflavin reacted with sodium nitroprusside in the presence of hydroxylamine hydrochloride and sodium carbonate in neutral medium giving rise to an intense greenish yellow colored solution. The color was stable for more than 24 h at room temperature. The wavelength at which the greenish yellow colored solution showed maximum absorbance ($\lambda_{max}$) was established by preparing an absorption spectrum in the wavelength region 350–500 nm both for the colored drug solution and reagent blank prepared by mixing all the solutions as described in experimental solution except the drug. The absorption spectrum plotted between wavelength and absorbance [figure not shown]. The figure showed that two peaks were observed one at 375 nm and another at 445 nm. However, the peak observed at 445 nm is having higher absorbance than the one at 375 nm. The reagent blank has shown no absorbance in the entire wavelength region studied. Hence, the analysis of riboflavin was carried out by measuring the absorption of the experimental solution at 445 nm against reagent blank.

Sodium nitroprusside is an Fe(II) complex which is surrounded by five cyanide and one nitric oxide ligands. In the presence of hydroxylamine hydrochloride and alkali, sodium carbonate it exists as ferrocyanide. The secondary amine group of riboflavin functions as an electron donar [5]. In the present mechanism, greenish yellow color formation is attributed to the reaction between ferrocyanide and riboflavin resulting in the formation of an inner complex with secondary nitrogen in the drug as donar atom. The greenish yellow color development was instantaneous and the color was stable for more than 4 h. The analytical data, i.e. coefficient of determination $r^2$=0.9996, detection limit 0.564 ppm, determination limit 1.690 ppm, and relative standard deviation 1.28% along with the slope 0.0217 and y-intercept 0.011 of the calibration plot indicate that the method is in accordance with the proposed analytical method and is quite suitable for the determination of riboflavin in various drug formulations.

## 4. CONCLUSION

In day-to-day life pharmaceutical industry plays a key role in manufacturing new drugs. As the vitamins are major micronutrients in every human-being life, I was eagerly interested to research the studies of vitamins after the review of the past work from great scholars and has been chosen the topic for further studies. Thus, the proposed method is simple, sensitive, accurate, and rapid method in pharmaceutical formulations and can be extended in bulk dosage forms.

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****Bibliographical Sketch***

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