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NOVEL SPECTROSCOPIC METHODS FOR THE DETERMINATION OF RIBOFLAVIN IN BULK DOSAGE FORMS AND FORMULATIONS

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ABSTRACT
Sophisticated analytical methods viz. HPLC, Mass spectroscopy, which being employed for analysis is relatively expensive and hence need for a simple analytical method arises. In the proposed research work, such simple methods employing UV-Visible spectroscopy, have been developed and applied for routine determination of Riboflavin in pharmaceutical formulations and bulk dosage forms. These methods were based on the formation of colored species on binding of riboflavin with thymol blue in the presence of chloroform for method A and the formation of colored species on binding of the same compound with Ferric chloride in the presence of HCl for method B . The colored chromogens obtained in each method was measured spectrophotometrically with λmax at 440 nm (method A) and 450 nm (method B) respectively. Statistical and regression analysis of these methods exhibited Sandell's Sensitivity of 0.010 (for method A) and 0.0117 (method B) and the relative standard deviation (RSD) of these methods were found equal to 1.48 (method A) and 1.70 (method B) respectively, indicating that these methods developed for riboflavin are reproducible, for its determination in formulations and bulk dosage forms.

Keywords: Riboflavin, Spectroscopy, Molar Absorptivity, Beer's Law, Recovery.

INTRODUCTION

Riboflavin[7,8-Dimethyl-70-[(2s,3s,4r)-2,3,4,5-tetrahydroxypentyl]Benzo[g]pteridine-2,4-dione, also known as "Vitamin B_2" is required for a wide variety of cellular processes. It is an easily absorbed water soluble vitamin with a key role in maintaining health in humans and animals, and in energy metabolism, it is also essential for the metabolism of fats, ketone bodies, carbohydrates, and proteins. It is the central component of the cofactors FAD and FMN, and is therefore required by all flavono proteins. It is also used as an orange-red food colour additive, designated in Europe as the E number E101. Milk, cheese, leafy green vegetables, liver, kidneys, legumes, etc. are good sources of vitamin B2. 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 colour to the oxidized molecule. Vitamin B_2 was necessary for preventing pellagra. Riboflavin is also needed to help the body change vitamin B_6 and folate into forms it can use. It is also important for body growth and red blood cell production. Riboflavin is effective for preventing and treating riboflavin deficiency and conditions related to riboflavin deficiency. Riboflavin is possibly effective for preventing migraine

headaches. Taking high-dose riboflavin (400mg/day) seems to significantly reduce the number of migraine headache attacks. However, taking riboflavin does not appear to reduce the amount of pain or the amount of time a migraine headache lasts.

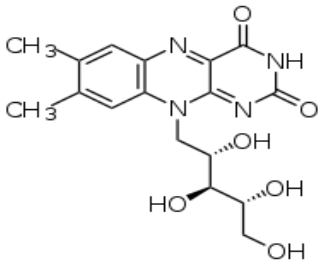

Fig 1: Structure of Riboflavin

Among the various methods available for the determination of Riboflavin like HPLC[6-10], Capillary Electrophoresis[11-13], Capillary Zone Electrophoresis[14], Mass spectroscopy[15], Micellar electro kinetic chromatography (MEKC)[16] and spectrophtometry[17-19], spectrophotometry continues to be very popular, because of its simplicity in operation, specificity of the equipment and low cost. The present paper proposes novel spectrophotometric methods for the determination of riboflavin and its further recovery from barley. No UV spectrophotometric methods have been reported for estimation of Riboflavin in single Component formulation by using complex formation of riboflavin with thymol blue and chloroform or reaction of riboflavin with ferric chloride and HCl. Hence, an attempt has been made to develop two methods for the estimation of riboflavin in pharmaceutical formulations with good accuracy, simplicity, precision and economy. The proposed methods for Riboflavin determination have many advantages over other analytical methods due to its rapidity and environmental safety. Economically, all the analytical reagents are inexpensive and available in any analytical laboratory. These methods can be extended for the routine quality control analysis of pharmaceutical products containing riboflavin.

EXPERIMENTAL

Instrumentation: After due calibration of the instrument, spectral and absorbance measurements were made using UV-Visible spectrophotometer model SL-159, Mumbai, India. All the chemicals used were of Analytical grade. All the solutions were freshly prepared using millipore double distilled water.

Standard and Sample solution of Riboflavin: About 100mg of Riboflavin was accurately weighed on a digital single pan balance and dissolved in a volumetric flask containing 100ml of water to prepare a standard solution with a concentration equal to 1mg/ml and further dilutions are made with the same solvent for this method.

Preparation of Reagents: All the chemicals used were of analytical grade. All solutions were freshly prepared with distilled water and always freshly used for analysis. Aqueous solutions of thymol blue (0.1%) and Chloroform(10ml) were used for method A, and Ferric chloride(0.1%) and HCl (1N for **Method-B**) were used for analysis.

ASSAY PROCEDURE

Method A: Into a series of separation flasks, aliquots of 0.4-2.0 ml of standard ribolavin solution were taken and the solution was made upto 2 ml with distilled in all the separation flasks. Riboflavin (0.5ml) and chloroform (10 ml) was added to each separation flask. The solutions were thoroughlly mixed in the separation flasks and the lower layer of puple colored solution was collected for spectroscopic measurements. Absorbance of the purple colored chromogen was measured at 440nm against the reagent blank.

Method B: Into a series of 5 test tubes, aliquots of 0.4-2.0ml of standard riboflavin solution was added and the solution was made upto 2ml with distilled in all the test tubes, Ferric chloridde (1.5ml) and HCl (1N, 0.5 ml) as added in each tube . the solution was finally made up to 5.0 ml with distilled water in all the test tubes. The absorbance of the resulting yellow colored chromogen was measured at 450nm against the reagent blank.

RESULTS AND DISCUSSION

The results of analysis for method A and B were validated through systematic statistical analysis and results are tabulated. The statistical analysis values are reported in Table -1 and assay and recovery results for these methods are tabulated in Table-2.

Method A: The results obtained in this method were due to ion association complex formation between the vitamin riboflavin and thymol Blue and Chloroform to form a purple colored solution that exhibited maximum absorption at 440nm against the corresponding reagent blank.

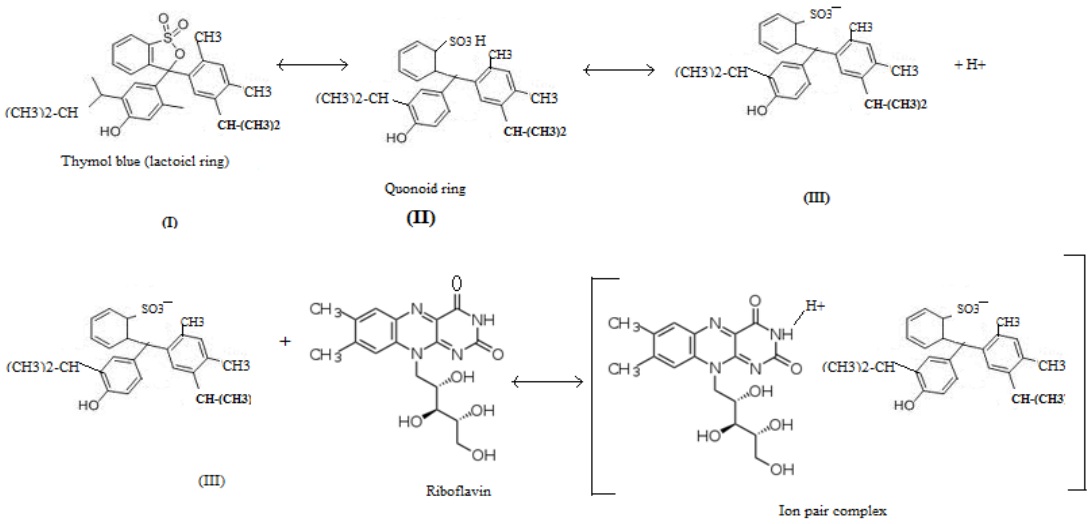

Fig.2. Mechanism of reaction between riboflavin and Thymol blue

Method B: The results obtained in this method were due to oxidation followed by complex formation under acidic conditions between the riboflavin and ferric chloride in the presence of HCl to form a yellow colored solution that exhibited maximum absorption at 450nm against the corresponding reagent blank.

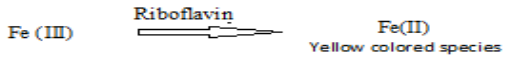

Fig: 3. Mechanism of reaction between riboflavin and ferric chloride

Table 1. Optical and Regression characteristics, Precision and accuracy of the Proposed methods for Riboflavin

Parameter	**Method A**	**Method B**
Name of the method	Thymol blue, chloroform	Ferric chloride, HCL
λ_{max} (nm)	440	450
Beer's law limits (µg / ml)	0.4-2.0	0.4-2.0
Molar absorptivity (L. mole^{-1} cm^{-1})	3.585	3.189
Sandell's sensitivity(µg /cm ^{2}/0.001 absorbance unit)	0.010	0 .0117
Regression equation (Y = a+ bc)*;Slope (b)	-423.901	-62.0
Intercept (a)	-50867.289	7439.19
Correlation coefficient (r)	9998.52	9998.30
Standard deviation**	0.0112	0.0110
% Relative standard deviation	1.48	1.70
% Range of Error (Confidence limits)		
0.05 level	±1.237	±1.421
0.01 level	±1.830	± 2.103

* Y= a + bx, where'Y'is the absorbance and x is the concentration of Riboflavin in µg/ml

** For four replicates.

Recovery: Barley is a major cereal grain, a member of the grass family. It serves as a major animal fodder, a source of fermentable material for beer and certain distilled beverages, and as a component of various health foods. It is used in soups and stews, and in barley bread of various cultures. Barley is a member of the grass family. It is a self-pollinating, diploid species with 14 chromosomes. The wild ancestor of domesticated barley, Hordeum vulgare subsp. spontaneum, is abundant in grasslands and woodlands throughout the Fertile Crescent and is abundant in disturbed habitats, roadsides and orchards. Outside this region, the wild barley is less common and is usually found in disturbed habitats. It is a widely adaptable crop. It is currently popular in temperate areas where it is grown as a summer crop and tropical areas where it is sown as a winter crop. Its germination time is anywhere from 1 to 3 days. Barley likes to grow under cool conditions but is not particularly winter hardly.

Preparation of standard solution: For recovery of Riboflavin bulk, we used barley water (100gms of barley is taken soaked in water for 4hrs. Then soaked water is used for recovery). Freshly prepared Standard solutions were used for analysis. In the proposed recovery methods Barley as taken in two concentrations 50 µg, 70 µg and diluted with 100ml of water separately. 1ml of each concentration was taken in two different test tubes for methods A and B.

Procedures for Recovery of Riboflavin:

Recovery with Method A: Into a Volumetric flask; Thymol Blue (2ml) was taken. To develop the initial color, chloroform (10ml), Riboflavin solution (2ml) was added to it and mixed well, resulting in formation of two layers. The lower layer was filtered in a clean test tube. Aliquots of 0.4-2.0 ml of standard Riboflavin solution were transferred to five different test tubes and made up to 2ml by adding water. Absorbance of the purple color chromogen was measured at 440nm against the reagent blank for method A

Recovery with Method B: To each test tube 1ml of Ferric chloride was added. To develop the initial color, Hydrochloric acid solution (2ml) was added to each tube respectively. After 5-10 min, absorbance of the yellow colored chromogen was measured at 450 nm against the reagent blank for method B.

Table 2.Assay and Recovery of Riboflavin in dosage forms

Biological Sample (riboflavin formulation) and (barley) (µg/ml)	Amount of riboflavin added to biological sample	Amount found		% Recovery by the developed methods	
		Method A	Method B	Method A	Method B
Sample - 1	50 µg	48	48	96.00	96.00
Sample - 2	70 µg	67	67	95.70	95.70

CONCLUSION

Performance recovery experiments and percent recovery values obtained in this work indicated the absence of interferences from commonly encountered pharmaceutical additives and excipients. The developed methods are simple and sensitive with reasonable precision and accuracy and can be employed as standard methods for the routine determination of Riboflavin in quality control analysis.

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